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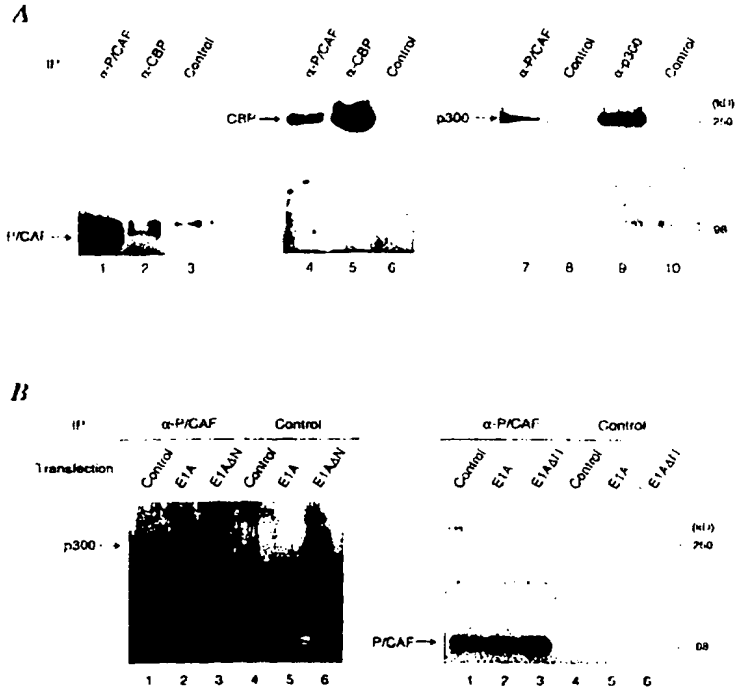
(57) Abstract

The present invention provides a purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones and which also binds to the p300/CBP cellular protein. The present invention further provides a nucleic acid encoding the P/CAF protein as well as a vector containing the nucleic acid and a host for the vector. A purified antibody which specifically binds the P/CAF protein is also provided. Also provided are methods of screening for compounds that inhibit or stimulate the transcription modulating and histone acetyltransferase activity of P/CAF and p300/CBP.



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P300/CBP-ASSOCIATED TRANSCRIPTIONAL CO-FACTOR P/CAF AND USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention provides a transcriptional co-factor, p300/CBP-associated factor (P/CAF), which modulates transcription through binding to the cellular transcription co-factors p300 and CBP and through acetylation of histones. Also provided are methods for screening for the presence of P/CAF and for substances which alter the transcription modulating effect and growth regulatory activity of P/CAF.

Background Art

Cellular proteins p300 and CBP are global transcriptional coactivators that are involved in the regulation of various DNA-binding transcriptional factors (Janknecht and Hunter, 1996). Recently, p300 was found to be very closely related to CBP, a factor that binds selectively to the protein kinase A-phosphorylated form of CREB (3-5). Cellular factors p300 and CBP exhibit strong amino acid sequence similarity and share the capacity to bind both CREB and E1A (6-8). Although neither p300 nor CBP by itself binds to DNA, each can be recruited to promoter elements via interaction with sequence-specific activators and functions to be a transcriptional adaptor. For simplicity, p300 and CBP will be termed p300/CBP in the context of discussing their shared functional properties.

p300/CBP is a large protein consisting of over 2,400 amino acids, known to interact with a variety of DNA-binding transcriptional factors including nuclear hormone receptors (13,57), CREB (3,4, 7), c-Jun/v-Jun (9,11), YY1 (10), c-Myb/v-Myb (12,58), Sap-1a (59), c-Fos (11) and MyoD (60). DNA-binding factors recruit p300/CBP not only by direct but also indirect interactions through cofactors; for example, nuclear hormone receptors recruit p300/CBP directly as well as through indirect interactions, via SRC-1, which stimulates transcription by binding to various nuclear hormone receptors (13,61).

The transforming proteins encoded by adenovirus and several other small DNA tumor viruses disturb host cell growth control by interacting with cellular factors that normally function to repress cell proliferation. One of the most intensively studied of these viral proteins, the product of the adenovirus E1A gene, is itself sufficient for transformation (1). E1A transforming activity resides in two distinct domains, the targets of which include p300/CBP and products of the retinoblastoma (RB) susceptibility gene family (1,2). Interactions of E1A with p300/CBP and RB are thought to influence functionally distinct growth regulatory pathways, allowing the two domains to contribute additively to transformation (1).

The paradigm for how E1A and functionally related viral proteins perturb cell growth regulation derives in large part from studies on their interactions with RB (1,2). The molecular function of E1A is based on its capacity to interfere with cellular protein-protein interactions. Since both E1A and various cellular targets bind to a site in RB termed the pocket domain (2), E1A can competitively disrupt the complex formation between RB and its cellular targets.

The second cellular factor implicated in E1A-dependent transformation, p300, is believed to inhibit G0/G1 exit, to activate certain enhancers, and to stimulate differentiation (1,2). E1A inhibits the p300/CBP-mediated transcriptional activation of many promoters (14). In one case that has been examined, the complex of p300 and YY1, E1A inhibits transcription without disrupting the complex (10).

The present invention provides a cellular protein designated P/CAF which binds to p300/CBP and plays an important role in both transcription and cell cycle regulation associated with a histone acetyltransferase activity. The present invention also provides a histone acetyltransferase activity in the p300/CBP cellular protein, thus providing targets for modulating transcription and cell cycle regulation in cells.

SUMMARY OF THE INVENTION

The present invention provides a purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones and which also binds to the p300/CBP cellular protein.

The present invention further provides a nucleic acid encoding the P/CAF protein as well as a vector containing the nucleic acid and a host for the vector. A purified antibody which specifically binds the P/CAF protein is also provided.

In addition, also provided is a bioassay for screening substances for the ability to inhibit the transcription modulating activity of P/CAF and/or histone acetyltransferase activity, comprising contacting the substance with a system in which histone acetylation by P/CAF can be determined; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit the transcription modulating activity and/or histone acetyltransferase activity of P/CAF.

Furthermore, the present invention provides a bioassay for screening substances for the ability to inhibit the transcription modulating activity and/or histone acetyltransferase activity of P/CAF comprising contacting the substance with a system in which the p300 binding of P/CAF can be determined; determining the amount of p300 binding of P/CAF in the presence of the substance; and comparing the amount of p300 binding of P/CAF in the presence of the substance with the amount of p300 binding of P/CAF in the absence of the substance, a decreased amount of p300 binding of P/CAF in the presence of the substance indicating a substance that can inhibit the transcription modulating activity and/or histone acetyltransferase activity of P/CAF.

Also provided is a method for determining the amount of P/CAF in a biological sample comprising contacting the biological sample with a polypeptide comprising the amino acid sequence of SEQ ID NO:3 under conditions whereby a P/CAF/p300 complex can be formed; and determining the amount of the P/CAF/p300 complex, the amount of the complex indicating the amount of P/CAF in the sample.

The present invention additionally provides a method for determining the amount of P/CAF in a biological sample comprising contacting the biological sample with an antibody which specifically binds P/CAF under conditions whereby a P/CAF/antibody complex can be formed; and determining the amount of the P/CAF/antibody complex, the amount of the complex indicating the amount of P/CAF in the sample.

Also provided herein is an assay for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of P/CAF, comprising: contacting the substance with a system in which histone acetylation by P/CAF can be determined; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased or increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of P/CAF.

The present invention further provides an assay for screening substances for the ability to inhibit binding of P/CAF to p300/CBP comprising: contacting the substance with a system in which the P/CAF binding of P300/CBP can be determined; determining the amount of P/CAF binding of p300/CBP in the presence of the substance; and comparing the amount of binding of P/CAF to p300/CBP in the presence of the substance with the amount of binding of P/CAF to p300/CBP in the absence of the substance, a decreased amount of binding of P/CAF to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of P/CAF to p300/CBP.

In addition, an assay is provided for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of p300/CBP, comprising: contacting the substance with a system in which histone acetylation by p300/CBP can be determined; determining the amount of histone acetylation by p300/CBP in the presence of the substance; and comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, a decreased or increased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of p300/CBP.

Furthermore, the present invention provides an assay for screening substances for the ability to inhibit binding of a DNA-binding transcription factor to p300/CBP comprising: contacting the substance with a system in which the DNA-binding transcription factor binding of P300/CBP can be determined; determining the amount of DNA-binding transcription factor binding of p300/CBP in the presence of the substance; and comparing the amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance with the amount of binding of DNA-binding transcription factor to p300/CBP in the absence of the substance, a decreased amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of DNA-binding transcription factor to p300/CBP.

A method is also provided for inhibiting the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

Also provided in the present invention is a method for stimulating the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

Furthermore, the present invention provides a method for inhibiting the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

Finally, the present invention additionally provides a method for stimulating the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A-B. Fig 1A: P/CAF-p300/CBP interaction *in vivo*. Cell extract was immunoprecipitated with rabbit anti-P/CAF (lanes 1, 4, and 7), rabbit anti-CBP (lanes 2 and 5), and mouse anti-p300 (lane 9) antibodies. For controls, cell extract was precipitated with rabbit control IgG (lanes 3, 6, and 8) or mouse anti-HA monoclonal antibody (lane 10). The precipitates were analyzed by immunoblotting with anti-P/CAF (lanes 1-3), anti-CBP (lanes 4-6), and anti-p300 (lanes 7-10) antibodies. The positions of non-specific bands are indicated by asterisks. Fig. 1B: E1A inhibits the P/CAF-p300 interaction *in vivo*. Osteosarcoma cells were transfected with either control vector (lanes 1 and 4) or E1A- (lanes 2 and 5) or E1AΔN- (lanes 3 and 6) expression vectors. Extract from the transfected subpopulation was immunoprecipitated with anti-P/CAF (lanes 1-3) or control (lanes 4-6) IgG. The precipitates were analyzed by immunoblotting with anti-p300 and anti-P/CAF.

Figs. 2A-F. P/CAF and E1A mediate antagonistic effects on cell cycle progression. HeLa cells (ATCC accession number CCL 2) were transfected by electroporation with 7 μg of P/CAF-expression plasmid and/or 3 μg of the full-length or the N-terminally deleted (Δ2-36) E1A 12S-expression plasmid as indicated in the figure. These plasmids were constructed by subcloning FLAG-P/CAF and E1A cDNAs into

pCX (34) and pcDNAI (Invitrogen), respectively. All samples, in addition, contained 1 μg of sorting plasmid (pCMV-IL2R) (31) and carrier plasmid (pCX) to normalize the total amount of DNA to 11 μg. After transfection, cells were incubated in Dulbecco's modified Eagle's medium with 10% fetal bovine calf serum for 12 hours and subsequently labeled in medium containing 10 μM bromo-deoxyuridine (BrdU) for 30 min. Subsequently, the transfected subpopulation was purified by magnetic affinity cell sorting and nuclei were analyzed by dual parameter flow cytometry as described (32). Histograms show percentages of cells in G1 and S phases. Abscissa values represent fluorescence intensity of bound anti-BrdU antibodies in log scale.

Fig. 3. Histone acetyltransferase activity of P/CAF. Activity of hGCN5 (lanes 1 and 4) and P/CAF (lanes 2 and 5) that acetylates free histones (lanes 1-3) or histones in the nucleosome core particle (35) (lanes 4-6) was measured as described (36). Each reaction contains 0.3 pmol of affinity purified FLAG-hGCN5 or FLAG-P/CAF, 4 pmol of the histone octamer or the nucleosome core particle and 10 pmol of [1-^{14}C]acetyl-CoA. Note that the histone octamer dissociates into dimers or tetramers under assay conditions. Acetylated histones were detected by autoradiography after separation by SDS-PAGE. The bands corresponding to acetylated histones H3 and H4 are indicated by arrows.

DETAILED DESCRIPTION OF THE INVENTION

As used in the specification and in the claims, "a" can mean one or more, depending upon the context in which it is used.

P/CAF protein and fragments

The present invention provides a purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones. The P/CAF protein can also bind to the amino acid region of SEQ ID NO:3

(amino acid (aa) residues 1753 - 1966) of the cellular transcriptional factor, p300 (which has the complete amino acid sequence of SEQ ID NO:6 and the nucleotide sequence of SEQ ID NO:12), and the amino acid region of SEQ ID NO:6 (amino acid residues 1805 - 1854) of the cellular transcriptional factor, CBP (which has the complete amino acid sequence of SEQ ID NO:7 and the nucleotide sequence of SEQ ID NO:13). The P/CAF protein can be defined by any one or more of the typically used parameters. Examples of these parameters include, but are not limited to molecular weight (calculated or empirically determined), isoelectric focusing point, specific epitope(s), complete amino acid sequence, sequence of a specific region (e.g., N-terminus) of the amino acid sequence and the like.

For example, The P/CAF protein can consist of the amino acid sequence of SEQ ID NO:1 or the P/CAF protein can comprise the amino acid sequence of SEQ ID NO:2 which represents the carboxy terminal end of the P/CAF protein and contains the histone acetyltransferase activity, or the amino acid sequence of SEQ ID NO:4, which represents the amino terminal end of the P/CAF protein, containing the binding site for p300/CBP. Because the amino-terminal region is specific for P/CAF it can be used to define and identify P/CAF.

As used herein, "purified" refers to a protein (polypeptide, peptide, etc.) that is sufficiently free of contaminants or cell components with which it normally occurs to distinguish it from the contaminants or other components of its natural environment. The purified protein need not be homogeneous, but must be sufficiently free of contaminants to be useful in a clinical or research setting, for example, in an assay for detecting antibodies to the protein. Greater levels of purity can be obtained using methods derived from well known protocols. Specific methods for purifying P/CAF proteins are known in the art.

As will be appreciated by those skilled in the art, the invention also includes those P/CAF polypeptides having slight variations in amino acid sequence which yield polypeptides equivalent to the P/CAF protein defined herein. Such variations may arise

naturally as allelic variations (*e.g.*, due to genetic polymorphism) or may be produced by human intervention (*e.g.*, by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion and substitution mutants. Minor changes in amino acid sequence are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules. Substitutions may be designed based on, for example, the model of Dayhoff, *et al.* (37). These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations.

Modifications to any of the P/CAF proteins or fragments can be made, while preserving the specificity and activity (function) of the native protein or fragment thereof. As used herein, "native" describes a protein that occurs in nature. The modifications contemplated herein can be conservative amino acid substitutions, for example, the substitution of a basic amino acid for a different basic amino acid. Modifications can also include creation of fusion proteins with epitope tags or known recombinant proteins or genes encoding them created by subcloning into commercial or non-commercial vectors (e.g., polyhistidine tags, flag tags, myc tag, glutathione-S-transferase [GST] fusion protein, *xylE* fusion reporter construct). Furthermore, the modifications can be such as do not affect the function of the protein or the way the protein accomplishes that function (e.g., its secondary structure or the ultimate result of the protein's activity). These products are equivalent to the P/CAF protein. The means for determining the function, way and result parameters are well known.

Having provided an example of a purified P/CAF protein, the invention also enables the purification of P/CAF homologs from other species and allelic variants from individuals within a species. For example, an antibody raised against the exemplary human P/CAF protein can be used routinely to screen preparations from different humans for allelic variants of the P/CAF protein that react with the P/CAF protein-specific antibody. Similarly, an antibody raised against an epitope, for example, from a conserved amino acid region of the human P/CAF protein can be used to routinely screen for homologs of the P/CAF protein in other species. A P/CAF protein can be

routinely identified in and obtained from other species and from individuals within a species using the methods taught herein and others known in the art. For example, given the present sequence, the DNA encoding a conserved amino acid sequence can be used to probe genomic DNA or DNA libraries of an organism to predictably obtain the P/CAF gene for that organism. The gene can then be cloned and expressed as the P/CAF protein and purified according to any of a number of routine, predictable methods. An example of the routine protein purification methods available in the art can be found in Pei et al. (38).

A purified polypeptide fragment of the P/CAF protein is also provided. The term "fragment" as used herein regarding a P/CAF protein, means a molecule of at least five contiguous amino acids of P/CAF protein that has at least one function shared by P/CAF protein or a region thereof. These functions can include antigenicity, binding capacity, acetyltransferase activity and structural roles, among others. The P/CAF fragment can be specific for a recited source. As used herein to describe an amino acid sequence (protein, polypeptide, peptide, etc.), "specific" means that the amino acid sequence is not found identically in any other source. The determination of specificity is made routine by the availability of computerized amino acid sequence databases and sequence comparison programs, wherein an amino acid sequence of almost any length can be quickly and reliably checked for the existence of identical sequences. If an identical sequence is not found, the protein is "specific" for the recited source. For example, a P/CAF fragment can be species-specific (e.g., found in the P/CAF protein of humans, but not of other species).

A fragment of the P/CAF protein having histone acetyltransferase activity can consist of the amino acid sequence of SEQ ID NO:2. A fragment of the P/CAF protein which binds to the amino acid sequence of SEQ ID NO:3 on p300 and the amino acid sequence of SEQ ID NO:9 on CBP can consist of the amino acid sequence of SEQ ID NO:4. To the extent that these fragments are specific for P/CAF, they can be used to identify and define P/CAF.

An antigenic fragment of P/CAF protein is provided. An antigenic fragment has an amino acid sequence of at least about five consecutive amino acids of a P/CAF protein amino acid sequence and binds an antibody or elicits an immune response in an animal. An antigenic fragment can be selected by applying the routine technique of epitope mapping to P/CAF protein to determine the regions of the proteins that contain epitopes reactive with antibodies or are capable of eliciting an immune response in an animal. Once the epitope is selected, an antigenic polypeptide containing the epitope can be synthesized directly, or produced recombinantly by cloning nucleic acids encoding the antigenic polypeptide in an expression system, according to standard methods.

Alternatively, an antigenic fragment of the antigen can be isolated from the whole P/CAF protein or a larger fragment of the P/CAF protein by chemical or mechanical disruption. Fragments can also be randomly chosen from a known P/CAF protein sequence and synthesized. The purified fragments thus obtained can be tested to determine their antigenicity and specificity by routine methods.

Nucleic Acids Encoding P/CAF Protein

An isolated nucleic acid that encodes a P/CAF protein is also provided. As used herein, the term "isolated" means a nucleic acid separated or substantially free from at least some of the other components of the naturally occurring organism, for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids. The isolation of nucleic acids can therefore be accomplished by techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids (39). It is not contemplated that the isolated nucleic acids are necessarily totally free of all non-nucleic acid components or all other nucleic acids, but that the isolated nucleic acids are isolated to a degree of purification to be useful in clinical, diagnostic, experimental, or other procedures such as, for example, gel electrophoresis, Southern, Northern or dot blot hybridization, or polymerase chain reaction (PCR).

A skilled artisan in the field will readily appreciate that there are a multitude of procedures which may be used to isolate the nucleic acids prior to their use in other procedures. These include, but are not limited to, lysis of the cell followed by gel filtration or anion exchange chromatography, binding DNA to silica in the form of glass beads, filters or diatoms in the presence of high concentrations of chaotropic salts, or ethanol precipitation of the nucleic acids.

The nucleic acids of the present invention can include positive and negative strand RNA as well as DNA and can include genomic and subgenomic nucleic acids found in the naturally occurring organism. The nucleic acids contemplated by the present invention include double stranded and single stranded DNA of the genome, complementary positive stranded cRNA and mRNA, and complementary cDNA produced therefrom and any nucleic acid which can selectively or specifically hybridize to the isolated nucleic acids provided herein. Stringent conditions (further described below) are used to distinguish selectively or specifically hybridizing nucleic acids from non-selectively and non-specifically hybridizing nucleic acids.

An isolated nucleic acid that encodes a P/CAF protein can be species-specific (i.e., does not encode the P/CAF protein of other species and does not occur in other species). Examples of the nucleic acids contemplated herein include the nucleic acid of SEQ ID NO:10 as well as the nucleic acids that encode each of the P/CAF proteins or fragments thereof described herein. P/CAF proteins and protein fragments can be routinely obtained as described herein and their structure (sequence) determined by routine means including the methods as used herein.

P/CAF protein-encoding nucleic acids can be isolated from an organism in which they are normally found (e.g., humans), using any of the routine techniques. For example, a genomic DNA or cDNA library can be constructed and screened for the presence of the nucleic acid of interest using one of the present P/CAF protein-encoding nucleic acids as a probe. Methods of constructing and screening such libraries are well known in the art and kits for performing the construction and screening steps are

commercially available (for example, Stratagene Cloning Systems, La Jolla, CA). Once isolated, the nucleic acid can be directly cloned into an appropriate vector, or if necessary, be modified to facilitate the subsequent cloning steps. Such modification steps are routine, an example of which is the addition of oligonucleotide linkers, which contain restriction sites, to the termini of the nucleic acid (See, for example, ref. 39).

P/CAF protein-encoding nucleic acids can also be synthesized. For example, a method of obtaining a DNA molecule encoding a specific P/CAF protein is to synthesize a recombinant DNA molecule which encodes the P/CAF protein. For example, nucleic acid synthesis procedures are routine in the art and oligonucleotides coding for a particular protein region are readily obtainable through automated DNA synthesis. A nucleic acid for one strand of a double-stranded molecule can be synthesized and hybridized to its complementary strand. One can design these oligonucleotides such that the resulting double-stranded molecule has either internal restriction sites or appropriate 5' or 3' overhangs at the termini for cloning into an appropriate vector.

Oligonucleotides complementary to or identical with the P/CAF protein-encoding nucleic acid sequence can be synthesized as primers for amplification reactions, such as PCR, or as probes to detect P/CAF protein encoding nucleic acids by various hybridization protocols (e.g., Northern blot; Southern blot; dot blot, colony screening, etc.).

Double-stranded molecules coding for relatively large proteins can readily be synthesized by first constructing several different double-stranded molecules that code for particular regions of the protein, followed by ligating these DNA molecules together. For example, Cunningham, et al. (40), have constructed a synthetic gene encoding the human growth hormone by first constructing overlapping and complementary synthetic oligonucleotides and ligating these fragments together. See also, Ferretti, et al. (41), wherein synthesis of a 1057 base pair synthetic bovine rhodopsin gene from synthetic oligonucleotides is disclosed.

By constructing a P/CAF protein-encoding nucleic acid in this manner, one skilled in the art can readily obtain any particular P/CAF protein with modifications at any particular position or positions. See also, U.S. Patent No. 5,503,995 which describes an enzyme template reaction method of making synthetic genes. Techniques such as this are routine in the art and are well documented. DNA encoding the P/CAF protein or P/CAF protein fragments can then be expressed *in vivo* or *in vitro*.

The nucleic acid encoding the P/CAF protein can be any nucleic acid that functionally encodes the P/CAF protein. To functionally encode the protein (i.e., allow the nucleic acid to be expressed), the nucleic acid can include, but is not limited to, expression control sequences, such as an origin of replication, a promoter, regions upstream or downstream of the promoter, such as enhancers that may regulate the transcriptional activity of the promoter, appropriate restriction sites to facilitate cloning of inserts adjacent to the promoter, antibiotic resistance genes or other markers which can serve to select for cells containing the vector or the vector containing the insert, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites and transcription termination sequences as well as any other sequence which may facilitate the expression of the inserted nucleic acid.

Preferred expression control sequences are promoters derived from metallothionine genes, actin genes, immunoglobulin genes, CMV, SV40, adenovirus, bovine papilloma virus, etc. A nucleic acid encoding a P/CAF protein can readily be determined based upon the genetic code for the amino acid sequence of the P/CAF protein and many nucleic acid sequences will encode a P/CAF protein. Modifications in the nucleic acid sequence encoding the P/CAF protein are also contemplated. Modifications that can be useful are modifications to the sequences controlling expression of the P/CAF protein to make production of P/CAF protein inducible or repressible as controlled by the appropriate inducer or repressor. Such means are standard in the art (*see, e.g.*, ref. 39). The nucleic acids can be generated by means standard in the art, such as by recombinant nucleic acid techniques, as exemplified in the examples herein, and by synthetic nucleic acid synthesis or *in vitro* enzymatic synthesis.

After a nucleic acid encoding a particular P/CAF protein of interest, or a region of that nucleic acid, is constructed, modified, or isolated, that nucleic acid can then be cloned into an appropriate vector, which can direct the *in vivo* or *in vitro* synthesis of that wild-type and/or modified P/CAF protein. The vector is contemplated to have the necessary functional elements that direct and regulate transcription of the inserted nucleic acid, as described above. The vector containing the P/CAF nucleic acid or nucleic acid fragment can be in a host (e.g., cell or transgenic animal) for expressing the nucleic acid. The P/CAF protein or fragment thereof can thus be produced in a host system containing the expression vector and its functional activity as described herein can be demonstrated according to methods well known in the art.

There are numerous *E. coli* (*Escherichia coli*) expression vectors known to one of ordinary skill in the art useful for the expression of proteins. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteria, such as *Salmonella, Serratia*, as well as various *Pseudomonas* species. These prokaryotic hosts can support expression vectors which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence and have ribosome binding site sequences, for example, for initiating and completing transcription and translation. If necessary, an amino terminal methionine can be provided by insertion of a Met codon 5' and in-frame with the gene sequence. Also, the carboxy-terminal extension of the protein can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression can be used. There are several advantages to yeast expression systems. First, evidence exists that proteins produced in yeast secretion systems exhibit correct disulfide pairing. Second, post-translational glycosylation is efficiently carried out by yeast secretory systems. The *Saccharomyces cerevisiae* pre-pro-alpha-factor leader region (encoded by the *MFα-1* gene) is routinely used to direct

protein secretion from yeast (42). The leader region of pre-pro-alpha-factor contains a signal peptide and a pro-segment which includes a recognition sequence for a yeast protease encoded by the *KEX2* gene. This enzyme cleaves the precursor protein on the carboxyl side of a Lys-Arg dipeptide cleavage-signal sequence. The polypeptide coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This construct is then put under the control of a strong transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The protein coding sequence is followed by a translation termination codon which is followed by transcription termination signals. Alternatively, the polypeptide encoding sequence of interest can be fused to a second protein coding sequence, such as Sj26 or β-galactosidase, used to facilitate purification of the resultant fusion protein by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion protein is applicable to constructs used for expression in yeast.

Efficient post-translational glycosylation and expression of recombinant proteins can also be achieved in *Baculovirus* expression systems in insect cells.

Mammalian cells permit the expression of proteins in an environment that favors important post-translational modifications such as folding and cysteine pairing, addition of complex carbohydrate structures and secretion of active protein. Vectors useful for the expression of proteins in mammalian cells are characterized by insertion of the protein encoding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or methotrexate resistance for use as selectable markers. For example, the antigen and immunoreactive fragment coding sequence can be introduced into a Chinese hamster ovary (CHO) cell line using a methotrexate resistance-encoding vector. Presence of the vector RNA in transformed cells can be confirmed by Northern blot analysis and production of a cDNA or opposite strand RNA corresponding to the protein encoding sequence can be confirmed by Southern and Northern blot analysis, respectively. A number of other suitable host cell lines capable of secreting intact proteins have been developed in the art and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, and the like.

Expression vectors for these cells can include expression control sequences, as described above. The vectors containing the nucleic acid sequences of interest can be transferred into the host cell by well-known methods, which vary depending on the type of cell host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cell hosts.

Alternative vectors for the expression of protein in mammalian cells, similar to those developed for the expression of human gamma-interferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease Nexin I, and eosinophil major basic protein, can be employed. Further, the vector can include CMV promoter sequences and a polyadenylation signal available for expression of inserted nucleic acid in mammalian cells (such as COS7).

The nucleic acid sequences can be expressed in hosts after the sequences have been positioned to ensure the functioning of an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors can contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired nucleic acid sequences (*see*, e.g., U.S. Patent 4,704,362).

The nucleic acids produced as described above can also be expressed in a host which is a non-human animal to create a transgenic animal, containing, in a germ or somatic cell, a nucleic acid comprising the coding sequence for all or a portion of the P/CAF protein, as well as all of the other regulatory elements required for expression of the P/CAF protein-encoding sequence. The animal will express the P/CAF gene or portion thereof to produce the P/CAF protein or protein fragment and such expression can be detected by determination of a particular phenotype unique to the transgenic animal expressing the transferred nucleic acid.

The nucleic acid can be the nucleic acid of SEQ ID NO:10, a nucleic acid having a nucleotide sequence which encodes the P/CAF protein, a nucleic acid having a nucleotide sequence which encodes the protein of SEQ ID NO:1, as well as the nucleic acids that encode the proteins comprising the fragments of SEQ ID NOS:2 and 4.

The nucleic acids of the invention can contain substitutions or deletions which provide a particular phenotype of interest. For example, various deletions or base substitutions can be introduced into the nucleic acid encoding the P/CAF protein for the purpose of studying the effects of these particular deletions or substitutions on the transcription modulation activity of the P/CAF protein. These effects can be monitored by observation of such characteristics as growth and development of the animal, the ability to develop tumors, survival rates and the like. The gene construct introduced into the animal cells to produce the transgenic animal can contain any of the regulatory elements described above to modulate expression of the foreign genes. As used herein, the term "phenotype" includes morphology, biochemical profiles, changes in tumor formation and other parameters that are affected by the presence of the P/CAF protein.

The transgenic animals of the invention can also be used in a method for determining the effectiveness of administering a nucleic acid encoding a functional P/CAF protein to a subject in need of a functional P/CAF protein. First, a nucleic acid encoding a nonfunctional P/CAF protein can be introduced into the animal's cells and expressed to yield a characteristic phenotype. Then, using standard gene therapy techniques, a nucleic acid encoding a functional P/CAF protein can be introduced into the animal's cells and the effects on the animal's phenotypic characteristics can be determined.

Having provided and taught how to obtain a nucleic acid that encodes a P/CAF protein, an isolated nucleic acid that encodes a fragment of P/CAF protein is also provided. The nucleic acid encoding the fragment can be obtained using any of the methods applicable to the nucleic acid encoding the entire P/CAF protein. The nucleic acid fragment can encode a species-specific P/CAF protein fragment (e.g., found in the

P/CAF protein of humans, but not in the P/CAF proteins of other species). Nucleic acids encoding species-specific fragments of P/CAF protein are themselves species-specific or allele-specific fragments of the P/CAF gene.

Examples of fragments of a nucleic acid encoding a fragment of the P/CAF protein can include the nucleic acid sequences which encode the amino acid sequences of the fragments of SEQ ID NOS:2 or 4. The same routine computer analyses used to select these examples of fragments can be routinely used to obtain others. Fragments of P/CAF-encoding nucleic acids can be primers for PCR or probes, which can be species-specific, gene-specific or allele-specific. P/CAF-encoding nucleic acid fragments can encode antigenic or immunogenic fragments of P/CAF protein that can be used in therapeutic assays or screening protocols. P/CAF gene fragments can encode fragments of P/CAF protein having histone acetylase activity and/or p300/CBP binding activity as described above, as well as other uses that may become apparent.

An isolated nucleic acid of at least ten nucleotides that selectively hybridizes with the nucleic acid of SEQ ID NO:10 under selected conditions is provided. For example, the conditions can be PCR amplification conditions and the hybridizing nucleic acid can be a primer consisting of a specific fragment of the reference sequence or a nearly identical nucleic acid that hybridizes only to the exemplified P/CAF-encoding nucleic acid or allelic variants thereof.

The invention provides an isolated nucleic acid that selectively hybridizes with the P/CAF-encoding nucleic acid sequence of SEQ ID NO:10 under stringent conditions. The hybridizing nucleic acid can be a probe that hybridizes only to the exemplified P/CAF-encoding nucleic acid sequence. Thus, the hybridizing nucleic acid can be a naturally occurring species-specific allelic variant of the exemplified P/CAF gene. The hybridizing nucleic acid can also include insubstantial base substitutions that do not prevent hybridization under the stated stringent conditions or affect either the function of the encoded protein, the way the protein accomplishes that function (e.g., its

secondary structure) or the ultimate result of the protein's activity. The means for determining these parameters are well known.

As used herein to describe nucleic acids, the term "selectively hybridizes" excludes the occasional randomly hybridizing nucleic acids as well as nucleic acids that encode other known homologs of the P/CAF protein. The selectively hybridizing nucleic acids of the invention can have at least 70%, 73%, 78%, 80%, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementarity with the segment and strand of the sequence to which it hybridizes. This list is not intended to exclude percent complementarity values between these values. The nucleic acids can be at least 10, 15, 16, 17, 18, 20, 21, 23, 24, 25, 30, 35, 40, 50, 100, 150, 200, 300, 500, 550, 750, 900, 950, or 1000 nucleotides in length or any intervening length, depending on whether the nucleic acid is to be used as a primer, probe or for protein expression. The hybridizing nucleic acid can comprise a region of at least ten nucleotides (up to full length) that is completely complementary to a unique region of the nucleic acid to which it hybridizes.

The nucleic acid can be an alternative coding sequence for the P/CAF protein, or can be used as a probe or primer for detecting the presence of or obtaining the P/CAF protein. If used as primers, the invention provides compositions including at least two nucleic acids which selectively hybridize with different regions of the nucleic acid so as to amplify a desired region. Depending on the length of the probe or primer, it can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions.

For example, for the purpose of obtaining or determining the presence of a nucleic acid encoding the P/CAF protein, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (P/CAF DNA in a sample) should be at least enough to exclude hybridization with a nucleic acid from another species. The invention provides examples of these nucleic acids of P/CAF, so that the degree of complementarity required to distinguish selectively

hybridizing from nonselectively hybridizing nucleic acids under stringent conditions can be clearly determined for each nucleic acid. It should also be clear that the hybridizing nucleic acids of the invention will not hybridize with nucleic acids encoding unrelated proteins (hybridization is selective) under stringent conditions.

"Stringent conditions" refers to the washing conditions used in a hybridization protocol. In general, the washing conditions should be a combination of temperature and salt concentration chosen so that the denaturation temperature is approximately 5-20°C below the calculated T_m of the nucleic acid hybrid under study. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to the probe or protein encoding nucleic acid of interest and then washed under conditions of different stringencies. For example, the nucleic acid sequence of SEQ ID NO:10 was used as a specific radiolabeled probe for the detection of messenger RNA transcribed from the P/CAF gene by performing hybridizations under stringent conditions. The T_m of such an oligonucleotide can be estimated by allowing 2°C for each A or T nucleotide, and 4°C for each G or C. For example, an 18 nucleotide probe of 50% G+C would, therefore, have an approximate T_m of 54°C.

The invention provides an isolated nucleic acid that selectively hybridizes with the P/CAF gene shown in the sequence set forth as SEQ ID NO:10 under stringent conditions. The invention further provides an isolated nucleic acid complementary to the nucleotide sequence set forth in SEQ ID NO:10.

Antibodies to the P/CAF protein

A purified antibody and an antiserum containing polyclonal antibodies that specifically bind the P/CAF protein or antigenic fragment are also provided. The term "bind" means the well understood antigen/antibody binding as well as other nonrandom association with an antigen. "Specifically bind" as used herein describes an antibody or other ligand that does not cross react substantially with any antigen other than the one specified, in this case, an antigen of the P/CAF protein. Antibodies can be made as

described in Harlow and Lane (33). Briefly, purified P/CAF protein or an antigenic fragment thereof can be injected into an animal in an amount and in intervals sufficient to elicit a humoral immune response. Serum polyclonal antibodies can be purified directly, or spleen cells from the animal can be fused with an immortal cell line and screened for monoclonal antibody secretion, according to procedures well known in the art. Purified monospecific polyclonal antibodies that specifically bind the P/CAF antigen are also within the scope of the present invention. The antibodies of the present invention can bind the protein of claim 1, the protein of claim 2, the protein of claim 3 and/or the protein of claim 4, as well as any other proteins of the present invention.

A ligand that specifically binds the antigen is also contemplated. The ligand can be a fragment of an antibody, such as , for example, an Fab fragment which retains P/CAF binding activity, or a smaller molecule designed to bind an epitope of the P/CAF antigen. The antibody or ligand can be bound to a substrate or labeled with a detectable moiety or both bound and labeled. The detectable moieties contemplated within the compositions of the present invention include those listed above in the description of the diagnostic methods, including fluorescent, enzymatic and radioactive markers.

The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or therapeutic compound or both bound and conjugated. Such conjugation techniques are well known in the art. For example, conjugation of fluorescent, radioactive or enzymatic moieties can be performed as described in the art (33,43). The detectable moieties contemplated in the present invention can include fluorescent, radioactive and enzymatic markers and the like. Therapeutic drugs contemplated with the present invention can include cytotoxic moieties such as ricin A chain, diphtheria toxin, pseudomonas exotoxin and other chemotherapeutic compounds.

It is well understood by one of skill in the art that all of the above discussion regarding antibodies to P/CAF can also be applied with regard to production, characterization and use of antibodies which bind the p300/CBP protein or any of the DNA-binding transcription factors of this invention.

Measuring the P/CAF protein in a sample

The present invention also provides a method for determining the presence and thus the amount of P/CAF protein in a biological sample. As used herein, a biological sample includes any tissue or cell which would contain the P/CAF protein. Examples of cells include tissues taken from surgical biopsies, isolated from a body fluid or prepared in an *in vitro* tissue culture environment.

One example of determining the amount of P/CAF in a biological sample can comprise contacting the biological sample with a polypeptide comprising the amino acid sequence of SEQ ID NO:3 under conditions whereby a P/CAF/p300 complex can be formed; and determining the amount of the P/CAF/p300 complex, the amount of the complex indicating the amount of P/CAF in the sample. Determination of the amount of P/CAF/p300 complex can be accomplished through techniques standard in the art. For example, the complex may be precipitated out of a solution and detected by the addition of a detectable moiety conjugated to the p300 protein or by the detection of an antibody which binds p300 or the P/CAF protein, as taught in the Examples herein. Antibodies which bind p300 or the P/CAF protein can be either monoclonal or polyclonal antibodies and can be obtained as described herein. Detection of P/CAF/p300 complexes by the detection of the binding of antibodies reactive with p300 or the P/CAF protein can be accomplished using various immunoassays as are available in the art, as described below.

Alternatively, determination of the amount of P/CAF in a biological sample can comprise contacting the biological sample with a polypeptide comprising the amino acid sequence of SEQ ID NO:9 under conditions whereby a P/CAF/CBP complex can be formed; and determining the amount of the P/CAF/CBP complex, the amount of the complex indicating the amount of P/CAF in the sample. Determination of the amount of P/CAF/CBP complex can be accomplished through techniques standard in the art. For example, the complex may be precipitated out of a solution and detected by the addition of a detectable moiety conjugated to the CBP protein or by the detection of an antibody which binds either CBP or the P/CAF protein, as taught in the Examples

herein. Antibodies which bind CBP or the P/CAF protein can be either monoclonal or polyclonal antibodies and can be obtained as described herein. Detection of P/CAF/CBP complexes by the detection of the binding of antibodies reactive with CBP or the P/CAF protein can be accomplished using various immunoassays as are available in the art, as described below.

Another example of determining the amount of P/CAF in a biological sample comprises contacting the biological sample with an antibody which specifically binds P/CAF under conditions whereby a P/CAF/ antibody complex can be formed and determining the amount of the P/CAF/antibody complex, the amount of the complex indicating the amount of P/CAF in the sample. Antibodies which bind P/CAF can be either monoclonal or polyclonal antibodies and can be obtained as described herein. Determination of P/CAF/antibody complexes can be accomplished using various immunoassays as are available in the art, as described below.

Immunoassays such as immunofluorescence assays, radioimmunoassays (RIA), immunoblotting and enzyme linked immunosorbent assays (ELISA) can be readily adapted for detection and measurement of P/CAF in a biological sample. Both polyclonal and monoclonal antibodies can be used in the assays. Available immunoassays are well known in the art and are extensively described in the patent scientific literature. See, for example, U.S. Patent Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876.

Screening assays for P/CAF

The present invention also provides a bioassay for screening substances for the ability to inhibit the histone acetyltransferase activity of P/CAF comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance under conditions whereby histone acetylation by P/CAF can occur; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the

amount of histone acetylation by P/CAF in the absence of the substance, a decreased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit the histone acetyltransferase activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to inhibit the histone acetyltransferase activity of P/CAF can be added to this system and assayed for inhibiting ability.

The present invention also provides a bioassay for screening substances for the ability to inhibit the transcription modulating activity of P/CAF, comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance under conditions whereby histone acetylation by P/CAF can occur; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit the transcription modulating activity and cell cycle progression suppressing activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to inhibit the transcription modulating activity of P/CAF by interfering with the histone acetyltransferase activity of P/CAF can be added to this system and assayed for inhibiting ability.

Also provided in the present invention is a bioassay for screening substances for the ability to inhibit the binding of p300 to P/CAF, comprising contacting a system in which the binding of p300 to P/CAF can be determined, with the substance under conditions whereby the binding of p300 and P/CAF can occur; determining the amount of p300 binding to P/CAF in the presence of the substance; and comparing the amount of p300 binding to P/CAF in the presence of the substance with the amount of p300 binding to P/CAF in the absence of the substance, a decreased amount of p300 binding to P/CAF in the presence of the substance indicating a substance that can inhibit the binding of p300 to P/CAF. The binding of p300 to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the p300 protein comprising the amino acid sequence of SEQ ID NO:3 and P/CAF. Alternatively, the system can comprise a cell extract produced from cells producing both p300 and P/CAF. Determination of the binding of p300 to P/CAF can be carried out as taught herein.

Additionally provided in the present invention is a bioassay for screening substances for the ability to inhibit the binding of CBP to P/CAF, comprising contacting a system in which the binding of CBP to P/CAF can be determined, with the substance under conditions whereby the binding of CBP to P/CAF can occur; determining the amount of CBP binding to P/CAF in the presence of the substance; and comparing the amount of CBP binding to P/CAF in the presence of the substance with the amount of CBP binding to P/CAF in the absence of the substance, a decreased amount of CBP binding to P/CAF in the presence of the substance indicating a substance that can inhibit the binding of CBP to P/CAF. The binding of CBP to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the CBP protein comprising the amino acid sequence of SEQ ID NO:9 and P/CAF. Alternatively, the system can comprise a cell extract produced from cells producing both CBP and P/CAF. Determination of the binding of CBP to P/CAF can be carried out as taught herein.

The present invention further contemplates a bioassay for screening substances for the ability to stimulate the histone acetyltransferase activity of P/CAF comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, an increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can stimulate the histone acetyltransferase activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to stimulate the histone acetyltransferase activity of P/CAF can be added to this system and assayed for stimulating ability.

The present invention further contemplates a bioassay for screening substances for the ability to stimulate the transcription modulating activity of P/CAF comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, an increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can stimulate the transcription modulating activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples.

Thus, the compound to be tested for the ability to stimulate the transcription modulating activity of P/CAF by increasing the histone acetyltransferase activity of P/CAF can be added to this system and assayed for stimulating ability.

The present invention further provides a bioassay for screening substances for the ability to stimulate binding of p300 to P/CAF, comprising contacting a system in which the binding of p300 to P/CAF can be determined, with the substance under conditions whereby the binding of p300 to P/CAF can occur; determining the amount of p300 binding to P/CAF in the presence of the substance; and comparing the amount of p300 binding to P/CAF in the presence of the substance with the amount of p300 binding to P/CAF in the absence of the substance, an increased amount of p300 binding to P/CAF in the presence of the substance indicating a substance that can stimulate the binding of p300 to P/CAF. The binding of p300 to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the p300 protein comprising the amino acid sequence of SEQ ID NO:3 and P/CAF. Alternatively, the system can comprise a cell extract produced from cells producing both p300 and P/CAF. Determination of the binding of p300 to P/CAF can be carried out as taught herein.

Additionally provided in the present invention is a bioassay for screening substances for the ability to stimulate the binding of CBP to P/CAF, comprising contacting a system in which the binding of CBP to P/CAF can be determined, with the substance under conditions whereby the binding of CBP to P/CAF can occur; determining the amount of CBP binding to P/CAF in the presence of the substance; and comparing the amount of CBP binding to P/CAF in the presence of the substance with the amount of CBP binding to P/CAF in the absence of the substance, an increased amount of CBP binding to P/CAF in the presence of the substance indicating a substance that can stimulate the binding of CBP to P/CAF. The binding of CBP to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the CBP protein comprising the amino acid sequence of SEQ ID NO:9 and P/CAF. Alternatively, the system can comprise a cell

extract produced from cells producing both CBP and P/CAF. Determination of the binding of CBP to P/CAF can be carried out as taught herein.

Transcription modulating activity of P/CAF

The present invention contemplates a method for inhibiting the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity inhibiting amount of a substance in a pharmaceutically acceptable carrier. For example, the substance can be identified according to the protocols provided herein as one that can inhibit the transcription modulating activity of P/CAF by preventing the binding of P/CAF to p300/CBP or by inhibiting the histone acetyltransferase activity of P/CAF as well as by any other inhibitory mechanism as identified by the protocols provided herein. Inhibition of the transcription modulating activity of P/CAF in a subject is desirable, for example, to inhibit HIV TAT-mediated transcription and therefore, the method of the present invention can be used to treat HIV-infected subjects.

The substance can be in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the substance, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject.

The transcription modulating activity and/or histone acetyltransferase activity of P/CAF can be inhibited in a subject by administering to the subject a substance which binds p300/CBP at the P/CAF binding site or a substance which binds the P/CAF protein at the p300/CBP binding site, the ultimate result being that P/CAF and p300/CBP do not bind with one another and P/CAF cannot exert its transcription modulating and/or histone acetyltransferase effect. The substance can be a protein, such as an antibody which binds the P/CAF protein binding site at or near the p300/CBP

binding site, thereby preventing its binding or an antibody which binds the p300/CBP protein at or near the P/CAF binding site, thereby preventing its binding. The substance can also bind the histone acetyltransferase site on P/CAF or at the acetylation site on the histone, thereby preventing acetylation by P/CAF.

The substance which binds p300/CBP, the P/CAF protein or the histone and has the net effect of inhibiting the transcription modulating effect and or histone acetyltransferase activity of P/CAF in the cell can be delivered to a cell in the subject by mechanisms well known in the art.

Alternatively, a nucleic acid encoding a protein which binds either to p300/CBP or the P/CAF protein and has the net effect of inhibiting the transcription modulating effect and/or histone acetyltransferase activity of P/CAF in the cell can be delivered to a cell in the subject by gene transduction mechanisms well known in the art. For example, nucleic acid can be introduced by liposomes as well as via retroviral or adeno-associated viral vectors, as described below.

The substance which inhibits the transcription modulating effect and/or histone acetyltransferase activity of P/CAF can be an antisense RNA or an antisense DNA which binds the RNA or DNA of P/CAF, thereby preventing translation or transcription of the RNA or DNA encoding P/CAF and having the net effect of inhibiting the transcription modulating effect and/or histone acetyltransferase activity of P/CAF by inhibiting P/CAF production. The antisense RNA of the present invention can be generated from the nucleic acid of SEQ ID NO:14 (human) or SEQ ID NO:15 (mouse). Furthermore, the antisense DNA can be a phosphorothioate oligodeoxyribonucleotide having the nucleotide sequence of SEQ ID NO:16 (human) or of SEQ ID NO:17 (mouse). The mouse antisense RNA can be used to inhibit the activity of mouse P/CAF, having the nucleotide sequence of SEQ ID NO:18 and the amino acid sequence of SEQ ID NO:8. The present invention also contemplates an antisense nucleic acid sequence which can bind the DNA or RNA of any of the transcription factors or other proteins now known or later identified to bind P/CAF, thereby inhibiting expression of the gene products of

these proteins and having the net effect of inhibiting the transcription modulating effect and/or histone acetyltransferase activity of P/CAF.

The antisense nucleic acid can comprise a typical nucleic acid, but the antisense nucleic acid can also be a modified nucleic acid or a derivative of a nucleic acid such as a phosphorothioate analogue of a nucleic acid. The composition can comprise, for example, an antisense RNA that specifically binds an RNA encoded by the gene encoding the serum protein. Antisense RNAs can be synthesized and used by standard methods (62).

Antisense RNA can inhibit gene expression by forming an RNA/RNA duplex between the antisense RNA and the RNA transcribed from the target gene. The precise mechanism by which this duplex formation decreases the production of the protein encoded by the endogenous gene probably involves binding of complementary regions of the normal sense mRNA and the antisense RNA strand with duplex formation in a manner that blocks RNA processing and translation. Alternative mechanisms include the formation of a triplex between the antisense RNA and duplex DNA or the formation of an DNA-RNA duplex with subsequent degradation of DNA-RNA hybrids by RNAse H. Furthermore, an antigene effect can result from certain DNA-based oligonucleotides via triple-helix formation between the oligomer and double-stranded DNA which results in the repression of gene transcription. Regardless of the specific molecular mechanism, the present invention results in inhibition of expression of the P/CAF gene by the introduced and replicated DNA resulting in inhibition of the transcription modulating and/or histone acetyltransferase activity of P/CAF, by a reduction in the expression of the nucleic acid to which the antisense nucleic acid is hybridized, and therefore a reduction of the gene product from the targeted gene.

The antisense nucleic acid may be obtained by any number of techniques known to one skilled in the art. One method of constructing an antisense nucleic acid is to synthesize a recombinant antisense DNA molecule. For example, oligonucleotide synthesis procedures are routine in the art and oligonucleotides coding for a particular

protein or regulatory region are readily obtainable through automated DNA synthesis. A nucleic acid for one strand of a double-stranded molecule can be synthesized and hybridized to its complementary strand. One can design these oligonucleotides such that the resulting double-stranded molecule has either internal restriction sites or appropriate 5' or 3' overhangs at the termini for cloning into an appropriate vector. Double-stranded molecules coding for relatively large proteins or regulatory regions can be synthesized by first constructing several different double-stranded molecules that code for particular regions of the protein or regulatory region, followed by ligating these DNA molecules together. Once the appropriate DNA molecule is synthesized, this DNA can be cloned downstream of a promoter in an antisense orientation. Techniques such as this are routine in the art and are well documented.

An example of another method of obtaining an antisense nucleic acid is to isolate that nucleic acid from the organism in which it is found and clone it in an antisense orientation. For example, a DNA or cDNA library can be constructed and screened for the presence of the nucleic acid of interest. Methods of constructing and screening such libraries are well known in the art and kits for performing the construction and screening steps are commercially available (for example, Stratagene Cloning Systems, La Jolla, CA). Once isolated, the nucleic acid can be directly cloned into an appropriate vector in an antisense orientation, or if necessary, be modified to facilitate the subsequent cloning steps. Such modification steps are routine, an example of which is the addition of oligonucleotide linkers which contain restriction sites to the termini of the nucleic acid. General methods are set forth in Sambrook *et al.* (39).

The DNA that is introduced into the cell is in an expression orientation that is antisense to a corresponding endogenous DNA or RNA of the cells. For example, where an endogenous DNA comprises a gene which encodes for a particular protein, the introduced DNA is in an expression orientation opposite the expression of the endogenous DNA; that is the DNA operatively linked to a promoter is in an antisense expression orientation relative to the corresponding endogenous gene. The introduced DNA may be homologous to the entire transcribed gene or homologous to only part of

the transcribed gene. Alternatively, the sequence of the introduced DNA may be divergent to that of the endogenous DNA but only divergent to the extent that hybridization of the nucleic acids occurs, thereby preventing transcription. One skilled in the art can determine the maximum extent of this divergence by routine screening of antisense DNAs corresponding to an endogenous DNA of the cell. In this manner, one skilled in the art can readily determine which fragments, or alternatively the extent of homology of the fragments or the entire gene that is necessary to inhibit gene expression.

The antisense nucleic acids of the present invention can be made according to protocols standard in the art, as well as described in the Examples provided herein. The antisense nucleic acids can be administered to a subject according to the gene transduction protocols standard in the art, as described below.

The present invention also contemplates a method for stimulating the transcription modulating activity and/or histone acetyltransferase activity of P/CAF in a subject comprising administering to the subject a substance, in a pharmaceutically acceptable carrier, determined according to the methods taught herein, to have a stimulatory affect on the transcription modulating and/or histone acetyltransferase activity of P/CAF. The substance can be one which has been identified, according to the protocols provided herein, to stimulate histone acetyltransferase activity in P/CAF or promote binding of P/CAF to p300/CBP. The stimulation of the transcription modulation activity and/or histone acetyltransferase activity of P/CAF in a subject is desirable, for example, to activate tumor suppressor p53 (which promotes apoptosis) or to activate the muscle differentiation factor, MyoD. Thus, the method of the present invention can be employed to treat cancer and to promote muscle differentiation in conditions where muscle differentiation is desired. The substance can be delivered to a cell in the subject by mechanisms well known in the art.

Further contemplated in the present invention is a method for promoting binding of P/CAF to p300/CBP in a subject, comprising administering to the subject a substance

identified by the methods provided herein to promote binding of P/CAF to either p300 or CBP.

Additionally, a nucleic acid encoding a protein which stimulates the transcription modulating activity and/or histone acetyltransferase activity of P/CAF can be delivered to a cell in the subject by gene transduction mechanisms, as described below.

Also provided in the present invention is a method of inhibiting the cell cycle progression inducing effect of an oncoprotein which binds p300/CBP in a subject comprising transducing the cells of the subject with a vector comprising a nucleic acid encoding the P/CAF protein; inducing expression of the nucleic acid in the cell to produce the P/CAF in an amount which will allow the P/CAF gene product to replace the oncoprotein bound to p300/CBP, whereby the replacement of the oncoprotein bound to p300/CBP by the P/CAF gene product inhibits the cell cycle progression inducing effect of the oncoprotein. The oncoprotein which binds p300/CBP in the cell can be the adenovirus E1A oncoprotein.

A method for providing a functional P/CAF protein to a subject in need of the functional P/CAF protein is also provided, comprising transducing the cells of the subject with a vector comprising a nucleic acid encoding the P/CAF protein and inducing expression of the nucleic acid to produce the functional P/CAF protein in the cell, thereby providing the functional P/CAF protein to the subject. The transduction of the vector nucleic acid into the subject's cells can be carried out according to standard gene therapy protocols well known in the art (see, for example, U.S. Patent No. 5,339,346).

Screening assays for p300/CBP

The present invention also provides a bioassay for screening substances for the ability to inhibit the histone acetyltransferase activity of p300/CBP comprising contacting a system, in which histone acetylation by p300/CBP can be determined, with the substance under conditions whereby histone acetylation by p300/CBP can occur;

determining the amount of histone acetylation by p300/CBP in the presence of the substance; and comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, a decreased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can inhibit the histone acetyltransferase activity of p300/CBP. The acetylation of histones by p300/CBP can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P300/CBP protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to inhibit the histone acetyltransferase activity of p300/CBP can be added to this system and assayed for acetyltransferase inhibiting ability.

Also provided in the present invention is a bioassay for screening substances for the ability to inhibit the binding of a transcriptional factor to p300/CBP, comprising contacting a system in which the binding of a transcriptional factor to p300/CBP can be determined, with the substance under conditions whereby the binding of the transcriptional factor and p300/CBP can occur; determining the amount of transcriptional factor binding to p300/CBP in the presence of the substance; and comparing the amount of transcriptional factor binding to p300/CBP in the presence of the substance with the amount of transcriptional factor binding to p300/CBP in the absence of the substance, a decreased amount of transcriptional factor binding to p300/CBP in the presence of the substance indicating a substance that can inhibit the binding of a transcriptional factor to p300/CBP. The binding of a transcriptional factor to p300/CBP can be determined in a system, for example, which can include a cell free reaction mixture comprising a transcriptional factor which binds p300/CBP and p300/CBP. Alternatively, the system can comprise a cell extract produced from cells producing both a transcriptional factor which binds p300/CBP and p300/CBP. The transcriptional factor which binds p300/CBP can be selected from, but is not limited to

the group consisting of nuclear hormone receptors, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YYI, Sap-1a, c-Fos, MyoD and SRC-1, as well as any other transcriptional factor now known or later identified to bind p300/CBP. The screening assay of the present invention can also be used to identify substances which inhibit the binding of p300/CBP to other components to which it is known to bind, for example, P/CAF, $pp90_{RSK}$, TFIIB, E1A, SV40 large T antigen, as well as any other substances now known or later identified to bind p300/CBP. Determination of the binding of a transcriptional factor or other substance to p300/CBP can be carried out as taught in the Examples herein as well as by protocols described in the literature.

The present invention further contemplates a bioassay for screening substances for the ability to stimulate the histone acetyltransferase activity of p300/CBP comprising contacting a system, in which histone acetylation by p300/CBP can be determined, with the substance; determining the amount of histone acetylation by p300/CBP in the presence of the substance; and comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, an increased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can stimulate the histone acetyltransferase activity of p300/CBP. The acetylation of histones by p300/CBP can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the p300/CBP protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to stimulate the histone acetyltransferase activity of p300/CBP can be added to this system and assayed for stimulating ability.

The present invention further provides a bioassay for screening substances for the ability to stimulate binding of a component, which binds p300/CBP, to p300/CBP, comprising contacting a system in which the binding of the component to p300/CBP can

be determined, with the substance under conditions whereby the binding of the component to p300/CBP can occur; determining the amount of component binding to p300/CBP in the presence of the substance; and comparing the amount of component binding to p300/CBP in the presence of the substance with the amount of component binding to p300/CBP in the absence of the substance, an increased amount of component binding to p300/CBP in the presence of the substance indicating a substance that can stimulate the binding of the component to p300/CBP. The binding of the component to p300/CBP can be determined in a system, for example, which can include a cell free reaction mixture comprising the component and p300/CBP. Alternatively, the system can comprise a cell extract produced from cells producing both the component and p300/CBP. The component which binds p300/CBP can be any of the transcriptional factors or other proteins which are known or are identified in the future to bind p300/CBP, as set forth above. Determination of the binding of the component to p300/CBP can be carried out as taught in the Examples provided herein and according to protocols available in the literature.

Histone acetyltransferase activity of p300/CBP

A method for inhibiting the histone acetyltransferase activity of p300/CBP in a subject is provided in the present invention, comprising administering to the subject a histone acetyltransferase activity inhibiting amount of a substance in a pharmaceutically acceptable carrier. The mechanism of the inhibitory action of the substance can be the inhibition of the binding of a DNA-binding transcription factor, such as, for example, a nuclear hormone receptor, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YY1, Sap-1a, c-Fos, MyoD or SRC-1, to p300/CBP.

The histone acetyltransferase activity of p300/CBP can be inhibited in a subject by administering to the subject a substance which binds p300/CBP at the transcription factor binding site or a substance which binds the transcription factor protein at the p300/CBP binding site, the ultimate result being that the transcription factor and p300/CBP do not bind with one another and p300/CBP cannot acetylate histones.

The substance which binds either to the transcription factor or the p300/CBP protein and has the net effect of inhibiting the histone acetyltransferase activity of p300/CBP in the cell can be identified according to the screening methods provided herein and delivered to a cell in the subject by mechanisms well known in the art. The substance can be a protein, such as an antibody which binds the p300/CBP protein binding site at or near the DNA-binding transcription factor binding site, thereby preventing its binding or an antibody which binds the DNA-binding transcription factor at or near the p300/CBP binding site, thereby preventing its binding. The substance can also bind the histone acetyltransferase site on p300/CBP (aa 1195-1673 on p300 or aa 1174-1850 on CBP) or at the acetylation site on the histone, thereby preventing acetylation by p300/CBP.

Additionally, the substance can be a nucleic acid which can be expressed in the cell to produce a protein which inhibits the histone acetyltransferase activity of p300/CBP. For example, a nucleic acid encoding a protein which binds either to a transcription factor or the p300/CBP protein and has the net effect of inhibiting the histone acetyltransferase activity of p300/CBP in the cell can be delivered to a cell in the subject by gene transduction mechanisms well known in the art. For example, nucleic acid can be introduced by liposomes as well as via retroviral or adeno-associated viral vectors, as described below.

The substance which inhibits the histone acetyltransferase activity of p300/CBP can be an antisense RNA or an antisense DNA which binds the RNA or DNA of p300/CBP thereby preventing translation or transcription of the RNA or DNA encoding p300/CBP and having the net effect of inhibiting the histone acetyltransferase activity of P/CAF by inhibiting p300/CBP production. The antisense RNA or DNA of the present invention can be produced and introduced into cells according to the same methods as set forth above for P/CAF antisense nucleic acids.

The present invention also contemplates a method for stimulating the histone acetyltransferase activity of p300/CBP in a subject comprising administering to the

subject a histone acetyltransferase activity stimulating amount of a substance, in a pharmaceutically acceptable carrier, determined according to the methods taught herein, to have a stimulatory affect on the histone acetyltransferase activity of p300/CBP. The substance can exert a stimulatory effect by promoting the binding of a DNA-binding transcription factor of the present invention to p300/CBP. The substance can be delivered to a cell in the subject by mechanisms well known in the art. A nucleic acid encoding a protein which stimulates the transcription modulating activity of p300/CBP can be delivered to a cell in the subject by gene transduction mechanisms, as described below.

Gene transduction

In the methods described above which include gene transduction into cells (i.e., addition of exogenous DNA into cells), the nucleic acids of the present invention can be in a vector for delivering the nucleic acids to the site for expression of the P/CAF protein. The vector can be one of the commercially available preparations, such as the pGM plasmid (Promega). Vector delivery can be by liposome, using commercially available liposome preparations or newly developed liposomes having the features of the present liposomes. Additionally, vector delivery can be via a viral system, including, but not limited to, retroviral, adenoviral and adeno-associated viral systems. Other delivery methods can be adopted and routinely tested according to the methods taught herein.

The modes of administration of the liposome will vary predictably according to the disease being treated and the tissue being targeted. For example, for treating cancer in either the lung or the liver, which are both sinks for liposomes, intravenous delivery is reasonable. For other localized cancers, as well as precancerous conditions, catheterization of an artery upstream from the target organ is a preferred mode of delivery, because it avoids significant clearance of the liposome by the lung and liver. For cancerous lesions at a number of other sites (e.g., skin cancer, localized dysplasias), topical delivery is expected to be effective and may be preferred, because of its convenience.

Leukemias and other disorders involving dysregulated proliferation of certain isolatable cell populations may be more readily treated by *ex vivo* administration of the nucleic acid.

The liposomes may be administered topically, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally or the like, although intravenous or topical administration is typically preferred. The exact amount of the liposomes required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disease being treated, the particular compound used, its mode of administration and the like. Thus, it is not possible to specify an exact amount. However, an appropriate amount may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

Topical administration can be by creams, gels, suppositories and the like. *Ex vivo* (extracorporeal) delivery can be as typically used in other contexts.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLES

I. P/CAF studies.

Cloning and characterization of P/CAF protein.

In human cells, CBP binds to c-Jun in a phosphorylation-dependent manner in association with stimulation of transcription (9). In yeast, GCN4 is believed to be a c-Jun counterpart on the basis of similarities in DNA recognition (15) as well as the participation of both proteins in UV signaling pathways (16). Yeast genetic screening has led to the isolation of various cofactors for GCN4, including GCN5 (yGCN5), ADA2 (yADA2) and ADA3 (yADA3) (17-19). These factors are considered to function as a complex (or in a common pathway) based on genetic and protein-protein interaction studies (18-22). Finally, p300/CBP and yADA2 exhibit significant sequence similarity within a 50 amino acid region including a Zn^{2+} finger motif (3). Human counterparts to yGCN5, yADA2, or yADA3 that interact with p300/CBP to mediate transcriptional activation by c-Jun were searched for in various nucleotide sequence databases.

Comparison of the yGCN5 protein sequence with various databases (23) revealed significant similarities with the two randomly sequenced human cDNAs, ETS05039 (24) ($P=4.0\times10^{-15}$) and NIB2000-5R ($P=6.5\times10^{-9}$). Given that these cDNAs were truncated, human fetal liver and fetal brain cDNA libraries (Clontech) were screened with ETS05039 and NIB2000-5R, respectively and complete clones were isolated from the human fetal liver cDNA library. The complete sequences revealed that the ETS05039- and NIB2000-5R-derived clones are encoded by distinct genes but are highly related within the protein coding regions (68% identity at the DNA level; 75% identity and 86% similarity at the protein level). The former encodes an N-terminal region with no sequence similarity to any proteins in the databases besides the yGCN5-related C-terminal region, whereas the latter encodes only the yGCN5-related region. Given that p300/CBP-binding activity was observed in the former polypeptide as shown below, it was designated p300/CBP-associated factor (P/CAF), having the amino acid

sequence of SEQ ID NO:1 and the nucleotide sequence of SEQ ID NO:10 and the latter was named human GCN5 (hGCN5), having the amino acid sequence of SEQ ID NO:5 and the nucleotide sequence of SEQ ID NO:11.

Additionally, an RNA blot (Clontech) was hybridized with a random-primed probe made from the cDNA encoding P/CAF. RNA blotting indicated that transcripts detected by the P/CAF and hGCN5 cDNAs are ubiquitously expressed, but the former is most abundant in heart and skeletal muscle, whereas the latter is most abundant in pancreas and skeletal muscle.

P/CAF-p300/CBP interaction *in vitro*

The P/CAF binding site was presumed to reside in the C terminal one third of CBP (residues 1,678-2,442) because it was observed that this region, when fused to a DNA binding domain, activates transcription (4) in a manner repressed by coexpression of 12S E1A. This region was divided into 6 overlapping fragments and each was expressed in *E. coli* as a glutathione-S-transferase (GST) fusion protein. GST-CBP fusions were incubated with recombinant P/CAF protein and, subsequently, purified using glutathione-Sepharose. Co-purified P/CAF was detected by immunoblotting analysis.

To construct GST-fusions, various regions of CBP and p300 were amplified by PCR. A series of deletions of the CBP segment B was created by site-directed *in vitro* mutagenesis (30). These fragments were subcloned into pGEX-2T (Pharmacia). GST-fusions were expressed in *E. coli* and extracted with buffer B [20 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 10% glycerol, 1 mM AEBSF, 0.1% NP40, 10 µg/ml of aprotinin, 10 µg/ml of leupeptin, 1 µg/ml of pepstatin A, 1 mM DTT] containing 0.1 M KCl for these experiments. GST-CBP-segment B was purified by glutathione-Sepharose and phenyl-Sepharose chromatographic steps, P/CAF, hGCN5, and E1A were expressed as FLAG-fusions in Sf9 cells via baculovirus vectors and affinity-purified with M2-agarose (ref. 30; Kodak-IBI). For interaction, a crude *E. coli* extract containing 20 pmol of GST-fusion was incubated with 40-60 pmol of P/CAF or E1A in a total volume of 50 µl of

buffer B with 0.1 M KCl on ice for 10 min. Samples were further incubated with 10 µl (packed volume) of glutathione-Sepharose at 4°C for 30 min, washed four times with 200 µl of buffer B containing 0.1 M KCl, and eluted with 20 µl of buffer E [50 mM Tris-HCl (pH 8.0), 0.2 M KCl, 20 mM glutathione] for 60 min. Interacting proteins were detected by anti-FLAG immunoblotting or silver staining.

For p300 interactions, the segment spanning residues 1763-1966 (segment B') of p300, which is analogous to the CBP segment-B, was used. Twenty percent of the P/CAF and hGCN5 inputs and 100% of the E1A input were also analyzed. In the GST precipitation assays, almost identical amounts of the GST fusions were recovered in all samples. Interaction between P/CAF and CBP (segment B) was determined in the absence and in the presence of E1A. Control reactions with GST-CBP alone and without GST-CBP were also performed. Input proteins were analyzed.

Two CBP segments, A and B, interacted specifically with P/CAF. The stronger interaction was observed in the latter segment, which does not include the yADA2-like Zn^{2+} finger. Given that the CBP segment-B is well conserved in p300 (66% identity, 75% similarity), the binding of P/CAF to p300 *in vitro* was also analyzed. For this experiment, the p300 segment spanning residues 1763-1966, termed segment B', which is analogous to the CBP segment-B, was used. Like CBP, p300 interacted specifically with P/CAF. These studies demonstrated that P/CAF binds specifically to both p300 and CBP *in vitro*. In contrast to P/CAF, hGCN5 did not bind to CBP or p300.

These studies also demonstrated that the Zn^{2+} finger region of p300/CBP, which shares sequence similarity with yADA2, is not essential for the interaction with P/CAF. Cloning of a human structural homolog of yADA2, termed hADA2 (25) has revealed that, unlike the sequence similarity between p300/CBP and yADA2, which is restricted to a 50 amino acid region, hADA2 shares extensive similarity (30% identity, 52% similarity) to yADA2 over the entire protein sequence. Moreover, a computer search of the complete genomic sequence of *Saccharomyces cerevisiae* revealed that yeast does

not have counterparts of p300/CBP or P/CAF. Thus, the p300/CBP-P/CAF pathway may have been acquired during metazoan evolution.

Action of E1A *in vitro*

Previous reports indicated that E1A binds to both the p300 segment spanning residues 1767-1816 and the CBP segment spanning residues 1805-1854 (7). These interactions were reconfirmed in the present system; thus, both p300 and CBP segments covering the previously identified regions interacted with E1A.

For further mapping, a series of deletions was introduced within the CBP segment-B and tested for interactions with P/CAF and E1A. Deletions of residues 1801-1825 or 1824-1851 markedly reduced interactions with both P/CAF and E1A, whereas deletion of residues 1850-1878 did not affect these interactions. Furthermore, deletion of residues 1801-1851 completely abolished interactions with both P/CAF and E1A. These data indicate that residues 1801-1851 of CBP are critical for interaction with both P/CAF and E1A. Taken together with the evidence that CBP segment A (aa residues 1,678-1,880) also binds to these factors, the above findings demonstrate that P/CAF and E1A bind to the same or very closely spaced sites on CBP.

Evidence that both P/CAF and E1A recognize the same p300/CBP segments raises the possibility of direct competition between P/CAF and E1A for binding to p300/CBP. To test this possibility, a competition experiment was performed with the use of affinity purified recombinant proteins. The interaction of P/CAF with the CBP-segment B was progressively inhibited by the addition of increasing amounts of E1A. In contrast, no inhibition was caused by an E1A mutant which does not bind to p300/CBP (E1AΔN). Similar results were obtained with the p300-segment B', leading to the conclusion that P/CAF and E1A compete for the same binding sites in p300/CBP.

P/CAF-p300/CBP interaction *in vivo*

The *in vivo* interaction between P/CAF and p300/CBP was established by co-immunoprecipitation from a human osteosarcoma cell extract. Proteins in this extract were immunoprecipitated with rabbit anti-P/CAF, rabbit anti-CBP and anti-p300 antibodies. For controls, cell extract was precipitated with rabbit control IgG or mouse anti-HA monoclonal antibody. The precipitates were analyzed by immunoblotting with anti-P/CAF, anti-CBP and anti-p300 antibodies.

Osteosarcoma cells were transfected with either control vector or E1A- or E1AΔN-expression vectors. Extract from the transfected subpopulation was immunoprecipitated with anti-P/CAF or control IgG. The precipitates were analyzed by immunoblotting with anti-p300 and anti-P/CAF antibodies.

Rabbit anti-P/CAF antibody was raised to the P/CAF segment spanning residues 125-397 and purified by immunoaffinity chromatography (33). A mixture of monoclonal antibodies raised to the human p300 segment spanning residues 1572-2371 (5) and rabbit polyclonal antibodies raised to the mouse CBP segment spanning residues 2-23 (for immunoprecipitation) and 1736-2179 (immunoblotting) were purchased from Upstate Biotechnology. Approximately 2 x 10^7 human osteosarcoma U-2 OS cells (ATCC accession number HTB 96) were extracted with 10 ml of lysis buffer [25 mM HEPES-KOH (pH 7.2), 150 mM potassium acetate, 2 mM EDTA, 1 mM DTT, 1 mM AEBSF, 10 μg/ml of aprotinin, 10 μg/ml of leupeptin, 1 μg/ml of pepstatin A, 20 mM sodium fluoride, 0.1% NP40]. Two to 10 ml of extract were incubated with 2 μg of the respective antibody for four hours at 4°C. Fifty μl (packed volume) of protein-A Trisacryl (Pierce) were added and incubation was continued for two hours. The matrix was washed four times with 1 ml of the lysis buffer, then boiled in 2x SDS sample buffer. Human osteosarcoma U-2 OS cells were transfected with 20 μg of the indicated plasmid and 1 μg of sorting plasmid (pCMV-IL2R) (31). The transfected subpopulation was purified by magnetic affinity cell sorting (32). Extract from approximately 2 x 10^5 sorted cells was immunoprecipitated as described.

Anti-P/CAF antibody specifically detected a 95 kDa protein, which is very close to the calculated value for the full-length P/CAF, in the immunoprecipitates. Anti-P/CAF antibody co-immunoprecipitated both CBP and p300. Similarly, anti-CBP antibody also co-immunoprecipitated P/CAF. However, anti-p300 antibody did not co-immunoprecipitate P/CAF. This is most likely due to steric interference since the anti-p300 antibody was raised to the p300 segment spanning residues 1572-2371 which includes the P/CAF binding region. These data demonstrate that P/CAF forms complexes with both p300 and CBP *in vivo*.

Action of E1A *in vivo*

The *in vitro* experiments described herein indicate that P/CAF and E1A compete for the binding sites in p300/CBP. Thus, a study was conducted to determine whether E1A targets the endogenous interaction between P/CAF and p300. An E1A-expression vector was transiently transfected into human osteosarcoma cells and the transfected subpopulation was purified by cell sorting. Then, the interaction between P/CAF and p300 in transfected cells was examined by co-immunoprecipitation with anti-P/CAF antibody. The endogenous interaction of P/CAF with p300 was drastically inhibited by expression of E1A. On the other hand, no inhibition was observed by the E1A mutant lacking the p300 binding domain (E1AΔN), indicating that E1A disrupts the P/CAF-p300 complex *in vivo* through an interaction with p300.

Cell cycle regulation by P/CAF

Given that binding of P/CAF to p300/CBP is inhibited by E1A, experiments were performed to evaluate whether P/CAF, by binding to and forming a functional complex with p300, is involved in the regulation of entry into S phase. This possibility was addressed by examining whether transient expression of P/CAF would affect the rate of G1/S transit in HeLa cells. P/CAF negatively affected the distribution of cells between G1 and S phases in this assay.

HeLa cells were transfected by electroporation with 7 μg of P/CAF-expression plasmid and/or 3 μg of the full-length or the N-terminally deleted (Δ2-36) E1A 12S-

expression plasmid as indicated. These plasmids were constructed by subcloning FLAG-P/CAF and E1A cDNAs into pCX (34) and pcDNAI (Invitrogen), respectively. All samples, in addition, contained 1 μg of sorting plasmid (pCMV-IL2R) (31) and carrier plasmid (pCX) to normalize the total amount of DNA to 11 μg. After transfection, cells were incubated in Dulbecco's modified Eagle's medium with 10% fetal bovine calf serum for 12 h, and subsequently labeled in medium containing 10 μM bromo-deoxyuridine (BrdU) for 30 min. Subsequently, the transfected subpopulation was purified by magnetic affinity cell sorting and nuclei were analyzed by dual parameter flow cytometry as described (32).

The fraction of cells accumulating in S phase in control cultures was 23%, compared to 15% in P/CAF-transfected cells. This effect was reproducible in multiple independent experiments. In parallel experiments to verify the utility of this experimental protocol, plasmids encoding E2F-1, simian virus 40 small t, cyclin A or cyclin E increased the accumulation of cells in S phase, whereas plasmids encoding the cyclin-dependent kinase inhibitors p21 or p27 reduced the number of S phase cells.

On the basis of evidence that E1A and P/CAF compete for binding sites on p300, it seemed possible that cotransfection of P/CAF with E1A would oppose the mitogenic effect caused by E1A. As shown by the data herein, this is indeed the case. E1A alone has mitogenic activity in this experimental setting, while the E1A mutant lacking the p300 binding domain (E1AΔN) has very weak activity. Comparable expression levels between wild type and mutant E1A in the transfected cells were revealed by immunoblotting analysis with anti-E1A. Intriguingly, when P/CAF was cotransfected with E1A, the mitogenic activity of E1A was significantly counteracted by P/CAF. These results show that P/CAF and E1A mediate antagonistic effects on cell cycle progression.

In the course of assessing P/CAF activity, it was also revealed that p300 is able to inhibit cell cycle progression under the same assay conditions. These findings suggest

that P/CAF and p300, perhaps by forming a complex, act in concert to suppress cell cycle progression.

Histone acetyltransferase activity in P/CAF

Acetylation of the N-terminal histone tails has been considered to play a crucial role in accessibility of transcription factors to nucleosomal templates (26-27). Recently, yGCN5 has been identified as a histone acetyltransferase (28). On the basis of this information, intrinsic histone acetyltransferase activity in P/CAF and hGCN5 was examined. As substrates, the core histones (histones H2A, H2B, H3 and H4) and the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) were used.

Activity of hGCN5 and P/CAF that acetylates free histones or histones in the nucleosome core particle (35) was measured as described (36). Each reaction contained 0.3 pmol of affinity purified FLAG-hGCN5 or FLAG-P/CAF, 4 pmol of the histone octamer or the nucleosome core particle and 10 pmol of [1-^{14}C]acetyl-CoA. The histone octamer dissociated into dimers or tetramers under assay conditions. Acetylated histones were detected by autoradiography after separation by SDS-PAGE.

P/CAF and hGCN5 acetylated the core histones with almost the same efficiency. Both factors acetylated histones H3 and H4, but preferentially H3. In contrast, very weak or no acetylation by hGCN5 was detected in the nucleosome core particles. Remarkably, significant acetylation by P/CAF was observed in a nucleosomal context. Although all core histones are acetylated in the nucleus, P/CAF and hGCN5 did not acetylate histones H2A and H2B *in vitro*.

Direct function of P/CAF is likely to involve its intrinsic histone acetyltransferase activity. Although exact molecular mechanisms by which acetylation of core histones contribute to transcription remains undefined, acetylation of the histones is considered to play an important role in transcriptional regulation (26-27). The positively charged N-terminal tails of core histones are believed to affect nucleosome structure by interacting

with DNA at or near the nucleosome-spacer junction. Acetylation of the histone tails presumably destabilizes the nucleosome and facilitates access by regulatory factors. Likewise, there is a general correlation between the level of acetylation and transcriptional activity of nucleosomal domains. The findings of the present invention provide insights into the mechanisms of targeted histone acetylation.

Cellular factor p300/CBP binds to various sequence-specific factors that are involved in cell growth and/or differentiation, including CREB (3,4), c-Jun (9), Fos (11), c-Myb (12) and nuclear receptors (13). P/CAF could stimulate the activation function of these factors via promoter-specific histone acetylation. The present invention demonstrates that E1A appears to perturb normal cellular regulation by disrupting the connection between p300/CBP and its associated histone acetyltransferase.

II. p300/CBP studies.

Purification of E1A associated histone acetyltransferase.

FLAG-epitope tagged E1A (or ΔE1A) was expressed in Sf9 cells (ATCC accession number CRL 1711) by infecting recombinant baculovirus (43). All purification steps were carried out at 4°C. Extract was prepared from infected cells by one cycle of freeze and thaw in buffer B (20 mM Tris-HCl, pH 8.0; 5 mM $MgCl_2$; 10% glycerol; 1 mM PMSF; 10 mMβ-mercaptoethanol; 0.1% Tween 20) containing 0.1 M KCl and the complete protease inhibitor cocktail (Boehringer Mannheim). To prepare E1A-immobilized beads, the extract was incubated with M2 anti-FLAG antibody agarose (Kodak-IBI) for four hours with rotating and subsequently washed with the same buffer three times. The resulting beads were incubated with HeLa (ATCC accession number CCL 2) nuclear extract for four to eight hours and thereafter washed with the same buffer six times. Finally, FLAG-E1A was eluted from the beads along with associated polypeptides by incubating with the same buffer containing 0.1 mg/ml FLAG peptide.

For further purification, eluted polypeptides were dialyzed in 0.05 M KCl-buffer B and subsequently loaded onto a SMART Mono Q column (Pharmacia) equilibrated with the same 0.05 M KCl-buffer B. After washing, the column was developed with a linear gradient of 0.05-1.0 M KCl in buffer B. Mono Q fractions were concentrated with a MICROCON spin-filter (Amicon) and consequently loaded onto a SMART Superdex 200 column (Pharmacia) equilibrated with 0.1 M KCl-buffer B.

Histone acetyltransferase assays

Filter binding assays were performed as described (80) with minor modifications. Samples were incubated at 30°C for 10-60 minutes in 30 ml of assay buffer containing 50 mM Tris-HCl, pH 8.0; 10% glycerol; 1 mM DTT; 1 mM PMSF; 10 mM sodium butyrate; 6 pmol of [^{3}H]acetyl CoA (4.3 mCi/mmole, Amersham Life Science Inc.); and 33 mg/ml of calf thymus histones (Sigma Chemical Co.). In experiments where synthetic peptides were substituted for core histones, 50 pmol of each peptide were used. After incubation, the reaction mixture was spotted onto Whatman P-81 phosphocellulose filter paper and washed for 30 minutes with 0.2 M sodium carbonate buffer pH 9.2 at room temperature with 2-3 changes of the buffer. The dried filters were counted in a liquid scintillation counter.

PAGE analysis was done as above except that 90 pmol of [^{14}C]acetyl CoA (55 mCi/mmole, Amersham Life Science Inc.) and 9 pmol of core histones or mononucleosomes were used. Core histones and mononucleosomes were prepared as described (35). For trypsin digestion, reaction mixtures were further incubated with various amounts of trypsin on ice for 30 minutes. The samples were analyzed on one dimensional SDS-PAGE gels or two dimensional gels, where the first dimension was an acid-urea-PAGE gel (44) and the second dimension was an SDS-PAGE gel.

Protein expression

For baculovirus expression, cDNAs corresponding to p300 portions of aa 1-670, aa 671-1194 and aa 1135-2414 were amplified by PCR (EXPAND High Fidelity PCR System; Boehringer Mannheim) as KpnI-NotI fragments. The resulting fragments were

subcloned into a baculovirus transfer vector having the FLAG-tag sequence (43). The recombinant viruses were isolated using the BACULOGOLD system (Pharmingen), according to the manufacturer's protocol and were infected into Sf9 cells (ATCC accession number CRL 1711) to express FLAG-p300. Recombinant proteins were affinity purified with M2 anti-FLAG antibody-immobilized agarose (Kodak-IBI) according to the manufacturer's protocol.

For bacterial expression, cDNAs encoding the p300 portions and the CBP portion (aa 1174-1850) were first subcloned into the baculovirus transfer vector having the FLAG-tag as described above. Thereafter, the XhoI and NotI fragments encoding FLAG-p300 or FLAG-CBP fusions were resubcloned into the *E. coli* expression vector pET-28c (Novagene) digested with SalI and NotI. Recombinant proteins were expressed in *E. coli* BL21(DE3) and affinity purified with M2-antibody agarose.

Histone acetyltransferases that associate with E1A

Although the adenovirus E1A 12S protein (E1A) inhibits transcription in a variety of genes via direct binding to p300/CBP (45), E1A also stimulates transcription in some contexts (46). Thus, p300/CBP-bound E1A was tested to determine whether it might recruit histone acetyltransferases or deacetylases to regulate transcription. In addition, experiments were conducted as described below to determine if p300/CBP per se is a histone acetyltransferase.

Initially, recombinant FLAG-epitope tagged E1A was immobilized on anti-FLAG antibody beads. Immobilized E1A was incubated with a HeLa nuclear extract for affinity purification of E1A-associated polypeptides. FLAG-E1A was then eluted from the beads, along with E1A-associated polypeptides, by incubating with FLAG-peptide. Although E1A per se has no histone acetyltransferase activity, E1A recruited significant amounts of histone acetyltransferase activity from the nuclear extract. It is very unlikely that this activity is derived from P/CAF given that E1A and P/CAF cannot bind to p300/CBP simultaneously (43). Consistent with this, no P/CAF was detected in these fractions by immunoblotting.

The E1A N-terminus, a region that is not highly conserved among the various adenovirus serotypes, is involved in p300/CBP binding *in vivo*. Mutations in the N-terminal region lead to loss of the ability for p300/CBP binding without affecting RB binding (1,47). Thus, the requirement of the E1A N-terminal region for the recruitment of histone acetyltransferase activity was tested. In contrast to the wild type, the N-terminal deleted form of E1A (ΔN-E1A) recruited only a background level of acetyltransferase activity. In agreement with previous reports (47), the ΔN-E1A showed no ability to interact with p300/CBP, although it still retained the ability to interact with a variety of other polypeptides, including RB.

To define the relationship between p300/CBP and histone acetyltransferase activity, affinity purified E1A-binding polypeptides were separated by Mono Q ion-exchange column. Both p300/CBP and the acetyltransferase activity were coeluted at 140 mM KCl, while most of polypeptides were eluted at 260 mM KCl. The active fraction of Mono Q column (~140 mM KCl) was further separated by Superdex-200 gel filtration column. Both p300/CBP and the acetyltransferase activity coeluted after the void volume, indicating that p300/CBP is involved in the histone acetyltransferase activity.

p300 is a histone acetyltransferase

The data provided herein indicate that p300 per se, or a polypeptide(s) associated with p300, possesses histone acetyltransferase activity. To test the former possibility, the acetyltransferase activity of recombinant p300 was measured. p300 was divided into three fragments, each of which was expressed in and purified from Sf9 cells via a baculovirus expression vector. Histone acetyltransferase activity was readily detected in the C-terminal fragment containing amino acids 1135-2414, whereas no activity was found in the other fragments, demonstrating conclusively that p300 per se is a histone acetyltransferase.

p300/CBP-histone acetyltransferase domain

To map the histone acetyltransferase domain of p300, a series of deletions was prepared. Given the poor conservation of the glutamine-rich region (aa 1815-2414) in the *C. elegans* p300/CBP homolog (6), the p300 fragment encoding aa 1135-1810 was expressed in and purified from *E. coli*. Importantly, this candidate region of p300 (aa 1135-1810) showed significant histone acetyltransferase activity. For further mapping within this region, a series of N-terminal deletions was constructed. Deletion of 60 residues, resulting in a fragment containing aa 1195-1810, had no effect on the acetyltransferase activity, whereas the deletion of 185 residues, yielding a fragment comprising aa residues 1320-1810, completely eliminated the acetyltransferase activity.

Next, a series of C-terminal deletions was analyzed to determine the requirement of the P/CAF (or E1A) -binding domain. The p300 fragments lacking the E1A binding domain (aa 1195-1760, 1195-1706 and 1195-1673) still retained the acetyltransferase activity, whereas the further truncated mutant (aa 1195-1652) completely lost the acetyltransferase activity. Consistent with these results, the internal deletion of residues 1418-1720 showed no acetyltransferase activity. These data demonstrate that the histone acetyltransferase domain is located between the bromodomain and the E1A-binding domain. Given that the histone acetyltransferase domain is highly conserved between p300 and CBP (91% similarity), the corresponding region of CBP, aa residues 1174-1850, was expressed to confirm the acetyltransferase activity. As expected, comparable activity was detected, indicating that both p300 and CBP are histone acetyltransferases.

Among various acetyltransferases including histone acetyltransferases GCN5 and P/CAF, putative acetyl-CoA binding sites are conserved (48). However, multiple alignment analysis (49) showed that the p300/CBP histone acetyltransferase domain does not belong to this group. Moreover, comparison of the p300/CBP histone acetyltransferase domain with peptide sequence databases (23) showed no sequence similarity to any other proteins. Accordingly, this invention shows that p300/CBP represents a novel class of acetyltransferases in that it does not have the conserved motif found among previously described acetyltransferases (48).

p300 acetylates all core histones in mononucleosomes

Substrate specificity for acetylation by p300 was also examined. As substrates, histone octamers and mononucleosomes (146 base pairs of DNA wrapped around the octamer of core histones) were used. Given that the histone octamer dissociates into dimers or tetramers under physiological conditions, the histone octamer is referred to here as core histones. When core histones were used, p300 acetylated all four proteins, but preferentially H3 and H4. More importantly, in a nucleosomal context, p300 acetylated all four core histones nearly stoichiometrically. In contrast, p300 acetylated neither BSA nor lysozyme.

Hyperacetylated histones are believed to be linked with transcriptionally active chromatin (26,27,50,51). Hyperacetylated forms are found in histones H4, H3 and H2B, which have multiple acetylation sites *in vivo*. Thus, the level of acetylation by p300 was also tested.

Mononucleosomes treated with p300 were analyzed by two-dimensional gel electrophoresis. A Coomassie blue-stained gel and the corresponding autoradiogram showed that a significant amount of histones, especially H4, were hyperacetylated. Importantly, acetylation levels by p300 were very close to those of hyperacetylated histones prepared from HeLa nuclei treated with sodium butyrate, a histone deacetylase inhibitor. In contrast, no acetylated forms were detected in the reaction without p300. These results indicate that p300 acetylates histones in mononucleosomes to the hyperacetylated state by targeting multiple lysine residues.

p300 acetylates the four lysines in the histone H4 N-terminal tail *in vitro* which are acetylated *in vivo*

Lysines at positions 5, 8, 12 and 16 of histone H4 are acetylated *in vivo* (51). Recent studies with yeast histone acetyltransferases demonstrate the position-specific acetylation by distinct acetyltransferases, i.e., while cytoplasmic acetyltransferases for histone deposition and chromatin assembly modify

positions 5 and 12, GCN5 modifies positions 8 and 16 (52). Accordingly, the positions of acetylation by p300 were also determined. A series of synthetic peptides containing acetylated lysines at various positions was used to determine the acetylation site-specificity of p300. Consistent with the two-dimensional gel electrophoresis analysis, the experiments with peptide substrates showed that p300 acetylates all four lysines in the histone H4 that are acetylated *in vivo*. These results are consistent with the view that deposition-related diacetylated histones are deacetylated during maturation of chromatin (53).

p300 preferentially acetylates the N-terminal histone tail

Histone acetyltransferases modify specific lysine residues in the N-terminal tail of core histones but not the C-terminal globular domain *in vivo* (26,27,50,51). Structural models of nucleosomes (54,55,56) suggest that most of the lysine residues in the C-terminal globular domain are buried. Therefore, experiments were conducted to examine whether restricted acetylation of the N-terminal tail resulted from the substrate specificity of the enzyme or inaccessibility of the enzyme to the core domain in nucleosomes. The globular domains of all core histones contain a long helix flanked on either side by a loop segment and short helix, termed the "histone fold" (54,55,56). The histone fold is involved in formation of the stable H2A-H2B and H3-H4 hetero-dimers, consisting of extensive hydrophobic contacts between the paired molecules. Therefore, it is likely that a histone monomer cannot fold properly, thereby increasing access of the histone acetyltransferase to the core domain. Based on these considerations, experiments were conducted to determine whether p300 acetylates free histone H4 in a N-terminal-specific manner.

Histone H4 was acetylated with p300 and subsequently the histone tail was removed by partial digestion with trypsin. The distributions of radioactivity between intact and core histones were compared. While the globular core histone domain was predominant at the higher trypsin concentrations, radioactivity was detected mostly in the intact histone. These data demonstrate that p300 preferentially acetylates the N-terminal tail of histone H4.

III. P/CAF interaction with MyoD

Tissue culture and transfection experiments

C_2C_{12} mouse cells (ATCC accession number CRL 1772) were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum (FBS) until they reached confluence. Differentiation was induced by switching medium to differentiation medium (DM), consisting of DMEM containing 2% horse serum. $C_3H/10T1/2$ fibroblasts (ATCC accession number CCL 226) were grown in DMEM supplemented with 10% FBS. Cells were transfected by the calcium phosphate precipitation method. Total amounts of transfected DNA were equalized by empty vector DNA. After 12 h incubation in medium containing the precipitated DNA, the cells were washed and incubated in fresh DMEM containing 10% FBS for an additional 24 h. Afterwards, differentiation was induced by incubating in DM for 36 to 72 h. Chloramphenicol acetyltransferase (CAT) assays were performed as previously described (64,69). The quantities of cell extracts used for CAT assays were normalized toβ-galactosidase activity by cotransfection of 1 mg of the β-galactosidase expression vector, pON260.

Expression vectors used for transfection experiments are as follows: pCX-P/CAF for P/CAF (43); pCMV-bp300 for p300 (65), pCMV-p300 (1869-2414) (64) and pCMV-p300 (1514-1922) (60) for p300 wild type and mutants; pE1A12S, pE1A12S R2G, pE1A12S D2-36 and pE1A12S D121-130 for E1A wild type and mutants (66,67,68); and pEMSV-MyoD for MyoD (64).

The antisense P/CAF RNA expression vector, pcDNA3 P/CAF-AS, was created as follows. The 2.5 Kb EcoRI-KpnI fragment containing the entire P/CAF open reading frame was isolated from pCX-P/CAF (43). This fragment was subcloned into the

EcoRI-KpnI sites of plasmid pcDNA3 (Invitrogen) so that the antisense P/CAF RNA is driven under the CMV promoter. Reporter genes employed were 4RE-CAT and MCK-CAT (69). 4RE-CAT is driven by a synthetic promoter containing 4 copies of the E-box, whereas MCK-CAT is driven by the native MCK promoter (nucleotides -1256 to +7).

Microinjection and immunofluorescence

Cells were grown on small glass slides, subdivided into numbered squares of 2 mm x 2 mm and microinjected with purified and concentrated antibodies, as previously described (70). For immunofluorescence, cells were fixed in either 2% paraformaldehyde or 1:2 methanol/acetone solution, preincubated with 5% BSA/PBS and incubated with the primary antibodies for 30 min at 37° C. Subsequently, antibody was visualized by incubating with either rhodamine- or fluorescein-conjugated secondary antibody for 30 min at 37° C. Injected antibodies were stained with a rhodamine-conjugated secondary antibody and nuclei were counter-stained by DAPI as previously described (69).

Antibodies employed are as follows; rabbit polyclonal affinity purified anti-P/CAF antibody (43), rabbit polyclonal anti-p300/CBP antiserum (71), mouse monoclonal anti-MyoD antibody (clone 5.8A, kindly provided by P. Houghton), goat polyclonal anti-c-Jun affinity purified antibody (Santa Cruz) and rabbit pre-immune serum.

Immunoprecipitation and DNA affinity purification

Cells were resuspended in lysis buffer (20 mM $NaPO_4$, 150 mM NaCl, 5mM $MgCl_2$, 0.1% NP40, 1 mM DTT, 10 mM sodium fluoride, 0.1 mM sodium vanadate, 1 mM phenylmethylsulfonyl-fluoride and 10 mg/ml each of leupeptin, aprotinin and pepstatin). After 30 min incubation on ice, samples were centrifuged at 12,000 x g for 30 min and supernatants were used as cell extracts. Extracts were pre-cleared by

incubating with rabbit pre-immune serum and protein A/G Plus-Agarose (Santa Cruz) for 2 h at 4 C. For immunoprecipitation, the supernatants were incubated with the respective antibodies for 3 h at 4 C. Protein A/G Plus-Agarose was added, and incubation continued for 3 h. The matrix was washed with lysis buffer, then boiled in 2 X SDS sample buffer. Immunoblotting was performed by using the ECL chemiluminescent detection kit (Amersham) according to the manufacturer's protocol.

Affinity purification of E-box-bound complexes was done as previously described (69). Briefly, 100 ng of the biotinylated double stranded DNA containing the E-box were immobilized on streptavidin-conjugated magnetic beads and incubated with 500 mg of cell extracts in the presence of poly dI-dC. After extensive washing, bound proteins were eluted with SDS sample buffer and analyzed by immunoblotting.

In vitro protein-protein interaction assays

The CBP-B fragment and its deletion derivatives were expressed as GST-fusions described previously (43). MyoD and E1A (43) were expressed as FLAG-fusion proteins in Sf9 cells via a baculovirus expression system and affinity-purified on M2 anti-FLAG antibody-agarose (Kodak-IBI). Crude E. coli extracts containing GST-fusions were incubated with various amounts of MyoD and/or E1A in 50 ml of buffer B (20 mM Tris-HCl, pH 8.0, 0.1 M KCl, 5 mM $MgCl_2$, 10% glycerol, and 0.1% Nonidet P-40) on ice for 10 min. GST-precipitation was performed as described (43). MyoD and E1A were detected by immunoblotting with anti-FLAG M2 antibody. For the interaction between P/CAF and MyoD, 1.5 pmol of FLAG-P/CAF and 15 pmol of FLAG-MyoD were incubated in 50 ml of buffer B on ice for 10 min. The mixture was further incubated with 2 mg of anti-P/CAF (43) or anti-hADA2 antibody for 60 min. The immunocomplexes were precipitated by incubation with 10 ml of protein A-Trisacryl (Pierce) and rotated for 1-4 hr at 4oC. The matrix was washed 4 times with 200 ml of buffer B and boiled in 10 ml of 2 X SDS sample buffer. The proteins were resolved on a 4%-20% gradient SDS-PAGE and subjected to immunoblotting with the anti-FLAG M2 antibody. The blot was developed with the SUPERSIGNAL chemiluminescent substrates (Pierce).

P/CAF coactivates muscle-specific transcription

P/CAF and MyoD were co-transfected into mouse C3H10T1/2 fibroblasts, and MyoD-mediated transcription was determined from reporter activity driven by the artificial (4RE) and the naturally-occurring muscle creatine kinase (MCK) promoters. Overexpression of P/CAF stimulated MyoD-dependent transcription several folds in both promoters. Similar results were obtained for the myoD activated myogenin promoter Transcriptional activation was further stimulated by co-transfecting with MyoD, P/CAF and p300 expression vectors, suggesting that P/CAF may function by forming a complex with p300/CBP. Consistent with the lack of DNA binding capacity in P/CAF, overexpression of P/CAF alone did not increase the basal transcriptional activity of either enhancer. To test whether P/CAF and p300/CBP function in the same pathway, two dominant negative forms of p300 were employed which specifically inhibit p300/CBP-mediated transcription (60,64). The p300 segment spanning residues 1514-1922 inhibits the MyoD-dependent activation via direct interaction with MyoD (60), whereas the p300 segment spanning residues 1869-2414 inhibit it without direct interaction (64). Both dominant negative mutants inhibited MyoD-coactivation by P/CAF), suggesting that P/CAF and p300/CBP function in the same pathway.

For further elucidation of the activation mechanism by P/CAF, the effect of E1A, which inhibits MyoD-dependent transcription and differentiation (66,72,73) via direct interaction with p300/CBP (65,78), was tested. Expression of E1A in C3H10T1/2 fibroblasts inhibited stimulation of MyoD-directed transcription by P/CAF overexpression. E1A mutants lacking p300/CBP-binding activity, E1A D2-36 and E1A R2G (67,79), had almost no effect. On the other hand, an E1A mutant retaining p300/CBP-binding activity, E1A D121-130, behaved like the wild type. Since E1A associates with p300/CBP, but not with P/CAF, these results suggest that P/CAF functions in MyoD-directed transcription via interaction with p300/CBP.

To address the role of P/CAF as a myogenic coactivator in a more relevant environment, P/CAF was overexpressed in proliferating C2C12 myoblasts which express

endogenous myogenic bHLH factors. As observed in fibroblasts, overexpression of P/CAF stimulated muscle specific transcription. Concomitant expression of exogenous p300 increased P/CAF-mediated coactivation. The repression exerted by wild type E1A, but not mutant E1A D2-36, on P/CAF coactivation of MyoD was also observed in muscle cells.

Similar experiments were performed with myogenic cell lines that were stably transformed with wild type or mutant E1A-expressing vectors (66). Coactivation by P/CAF was inhibited by wild type E1A or the E1A mutant that retains p300/CBP-binding activity (E1AΔ121-130). In contrast, E1A mutants that lack p300/CBP-binding (E1A Δ2-36 and E1A R2G) allowed transcriptional coactivation by P/CAF. Taken together, these experiments show that P/CAF coactivates MyoD-directed transcription via interaction with p300/CBP.

P/CAF stimulates myogenic differentiation

Given that P/CAF potentiates MyoD-directed transcription, the ability of P/CAF to assist MyoD in promoting myogenic differentiation was investigated. To this aim, C3H10T1/2 fibroblasts were transiently transfected with P/CAF and MyoD expression vectors. An expression vector for the green fluorescent protein (GFP) was co-transfected to identify transfected cells. After incubation in differentiation medium, the myogenic conversion of transfected cells was determined by simultaneous expression of the GFP and the differentiation-specific marker myosin heavy chain (MHC). Forced expression of MyoD in fibroblasts caused muscle differentiation in 12% of the transfected fibroblasts. This myogenic conversion was 20% by co-expressing MyoD and P/CAF. As observed in transcription experiments, stimulation of differentiation by P/CAF was counteracted by co-transfection with the p300 dominant negative mutant, p300 (1869-2414). Consistent with a general role for coactivators, overexpression of P/CAF alone was unable to differentiate fibroblasts.

Similar experiments were done using proliferating C2C12 myoblasts in which the differentiation program is already committed. Most of the myoblasts differentiated into

myotubes by overexpressing P/CAF, whereas only a modest effect was observed by overexpressing p300. In contrast, differentiation was inhibited slightly by overexpressing c-Jun. This inhibitory effect presumably was caused by titration of p300/CBP, which associates directly with c-Jun (74). A similar inhibition was observed in the p300 dominant negative mutant. Consistent with the transcriptional effect, E1A almost completely inhibited differentiation. The E1A mutant RG2, lacking p300/CBP-binding capability but retaining the retinoblastoma protein (Rb)-binding capability, only partially inhibited differentiation, although this same mutant
inhibited transcription as severely as the wild type. Taken together, these data show that P/CAF stimulates muscle differentiation by coactivating MyoD function via p300/CBP association.

P/CAF is essential for myogenic transcription and differentiation

To test the necessity of P/CAF for myogenic transcription, experiments were conducted whereby P/CAF synthesis was inhibited by expressing antisense P/CAF RNA. A vector from which the P/CAF mRNA is transcribed in the antisense orientation (P/CAF-AS) was transfected with P/CAF and MyoD expression vectors into fibroblasts and MyoD-dependent transcription was examined. Cotransfection of the antisense expression vector strongly inhibited MyoD-dependent transcription below the level of induction elucidated by MyoD alone, demonstrating that expression of P/CAF antisense RNA inhibits not only the coactivation exerted by exogenous P/CAF but also that of endogenous P/CAF. These results indicate that P/CAF is essential for MyoD-dependent transcription.

Studies were also carried out to determine whether expression of P/CAF antisense RNA inhibits myogenic differentiation. C3H10T1/2 fibroblasts were transiently transfected with various expression vectors with or without the P/CAF antisense RNA expression vector. Expression of P/CAF antisense RNA reduced MyoD-mediated myogenic conversion of fibroblasts. Expression of P/CAF antisense RNA also counteracted the stimulatory effect of both P/CAF and p300 on myogenic differentiation. These data support the view that P/CAF and p300/CBP coactivate

MyoD-dependent transcription in the same pathway. More drastic inhibition was observed in C2C12 myoblasts in similar experiments. Therefore, it can be concluded that P/CAF is essential for transcription of muscle specific genes and hence differentiation into myotubes.

To further confirm the essential role of P/CAF for myogenic differentiation, we blockage experiments by antibody microinjection were performed. Antibodies were injected into the cytoplasm of proliferating C2C12 myoblasts to prevent the nuclear transport of newly synthesized target proteins. After incubating in the differentiation medium, the degree of differentiation was determined. Microinjection of an anti-P/CAF antibody almost completely inhibited differentiation. Similar results were obtained by microinjecting anti-p300/CBP antibodies. Although microinjection of either anti-p300/CBP or P/CAF antibody was sufficient to inhibit differentiation, an even greater inhibition was observed by coinjecting both of them. Microinjection of anti-P/CAF or anti-p300/CBP antibody did not interfere with induction of p53 by DNA damaging agents, showing specificity of the inhibition by the antibodies. In contrast to anti-P/CAF or anti-p300/CBP antibodies, the injection of anti-MyoD antibody only partially inhibited differentiation, supporting the view of functional redundancy between MyoD and Myf-5 (75,76). Injection of anti-c-Jun antibody or control antibody did not interfere with muscle differentiation.

Similar experiments were performed with C3H10T1/2 fibroblasts stably expressing MyoD. In these cells, either anti-p300/CBP or anti-P/CAF antibody completely inhibited muscle differentiation. In contrast to myoblasts, anti-MyoD antibody completely blocked differentiation in the fibroblasts expressing MyoD. Anti-c-Jun and control antibodies did not interfere with differentiation. Taken together, these results demonstrate that P/CAF and p300/CBP are indispensable for activation of the myogenic program.

p300/CBP, P/CAF and MyoD form a multimeric complex *in vivo*

The data described above indicate that P/CAF stimulates MyoD-directed transcription via association with p300/CBP. Thus, experiments were conducted to investigate whether P/CAF, p300/CBP and MyoD could associate in a complex. First, cellular extracts derived from C2C12 myotubes were subjected to immunoprecipitation. Both anti-MyoD and anti-p300/CBP antibodies co-precipitated P/CAF. In a complementary experiment, both anti-p300/CBP and anti-P/CAF antibodies also co-precipitated MyoD, suggesting that these factors form a multimeric protein complex in myotubes.

Next, attempts were made to detect this complex on the E-box, the DNA binding site for MyoD. Immobilized DNA containing an E-box sequence was incubated with myotube extracts. After extensive washing, P/CAF, p300/CBP and MyoD were analyzed by immunoblotting. P/CAF, p300/CBP and MyoD were all affinity purified on the immobilized DNA, whereas they were not purified on the control DNA lacking the E-box. Given that P/CAF and p300/CBP per se cannot bind to DNA, these observations indicate that P/CAF and p300/CBP are recruited through MyoD at the E-box sites to form a multi-protein complex.

Complex formation is inhibited by viral transforming factors

Since the oncoviral proteins E1A and large T antigen inhibit myogenic transcription and differentiation, the effect of these factors on the formation of complexes on the E-box was tested. Importantly, very small amouts of P/CAF and p300/CBP were co-purified on the E-box from myocyte extracts which stably express E1A or large T antigen, although MyoD was detected under these conditions. The lower recovery of MyoD from E1A-expressing muscle cells could reflect the low level of MyoD in the extracts (66). These results indicate that E1A and large T antigen dissociate P/CAF and p300/CBP from MyoD without altering MyoD binding to DNA.

Consistent with the previous observations that transiently expressed E1A prevents interaction between P/CAF and p300/CBP *in vivo* (43), the association

between p300/CBP and P/CAF was abolished in myoblasts stably transformed by wild type E1A but not in those clones transformed with the E1A mutant R2G unable to bind p300/CBP. Similarly, the interaction between p300/CBP and P/CAF was abolished by large T antigen but not by the mutant protein that localizes into the cytoplasm (77).

Interaction between MyoD, P/CAF and CBP *in vitro*

Previous interaction experiments *in vitro* indicate that the CBP region spanning residues 1801 to 1850 is crucial for interaction with both P/CAF and E1A (43). While most sequence-specific factors bind to CBP sites distinct from the P/CAF/E1A binding sites, MyoD interacts with an overlapping CBP fragment called the CH3 region (60,64,65). To understand how P/CAF, p300/CBP and MyoD associate, the CBP sites important for MyoD binding were mapped more precisely. Consistent with previous reports (60,64,65), the CBP fragment spanning residues 1801-2000 (fragment B) bound MyoD. Moreover, deletion of residues 1801 to 1850 within fragment B completely abolished interaction with MyoD, which is similar to the results obtained with P/CAF and E1A. Importantly, an internal deletion of residues 1850-1878 abolished the MyoD interaction with CBP, while it did not affect binding of E1A or P/CAF (43). These results suggest that MyoD and P/CAF bind to distinct sites of p300/CBP, albeit the binding sites may overlap. Moreover, a direct interaction was observed between MyoD and P/CAF, which may contribute to stabilization of the multimeric complex.

These data show that E1A prevents not only p300/CBP-interaction with P/CAF but also that with MyoD *in vivo*. To obtain evidence that this inhibition is due to the direct action by E1A, competition experiments were performed *in vitro*. Importantly, the interaction between CBP and MyoD was strongly inhibited by addition of E1A, implicating that E1A inhibits myogenic transcription by disrupting multiple interactions.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be

regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Throughout this application various publications are referenced by numbers within parentheses. Full citations for these publications are as follows. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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SEQUENCE LISTING

(1) GENERAL INFORMATION.

(i) APPLICANT: The United States of America, as repesented by the Secretary, Department of Health and Human Services, c/o National Institutes of Health, Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, Rockville, Maryland 20842

(ii) TITLE OF THE INVENTION: METHODS AND COMPOSITIONS FOR p300/CBP-ASSOCIATED TRANSCRIPTIONAL CO-FACTOR P/CAF

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

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(B) FILING DATE: 23-JUL-1997
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: Corresponding U.S. Serial No. 60/022,273

(B) FILING DATE: 23-July-1996

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(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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        35                  40                  45
Ser Gly Ala Cys Gly Pro Ala Thr Ala Val Ala Ala Ala Gly Thr Ala
    50                  55                  60
Glu Gly Pro Gly Gly Gly Gly Ser Ala Arg Ile Ala Val Lys Lys Ala
65                  70                  75                  80
Gln Leu Arg Ser Ala Pro Arg Ala Lys Lys Leu Glu Lys Leu Gly Val
                85                  90                  95
Tyr Ser Ala Cys Lys Ala Glu Glu Ser Cys Lys Cys Asn Gly Trp Lys
            100                 105                 110
Asn Pro Asn Pro Ser Pro Thr Pro Pro Arg Ala Asp Leu Gln Gln Ile
        115                 120                 125
Ile Val Ser Leu Thr Glu Ser Cys Arg Ser Cys Ser His Ala Leu Ala
    130                 135                 140
Ala His Val Ser His Leu Glu Asn Val Ser Glu Glu Glu Met Asn Arg
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Ser Leu Glu Lys Lys Pro Pro Phe Glu Lys Pro Ser Ile Glu Gln Gly
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225                 230                 235                 240
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Tyr Trp His Leu Glu Ala Pro Ser Gln Arg Arg Leu Arg Ser Pro Asn
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Cys Asn Val Pro Gln Phe Cys Asp Ser Leu Pro Arg Tyr Glu Thr Thr
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Gln Val Phe Gly Arg Thr Leu Leu Arg Ser Val Phe Thr Val Met Arg
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        355                 360                 365
Phe Leu Ser Ala Ser Ser Arg Thr Ser Gln Leu Gly Ile Gln Thr Val
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Met Pro Lys Glu Tyr Ile Thr Arg Leu Val Phe Asp Pro Lys His Lys
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545                 550                 555                 560
Arg Met Phe Pro Ser Gln Gly Phe Thr Glu Ile Val Phe Cys Ala Val
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Thr Ser Asn Glu Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn His
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Ala Asp Glu Tyr Ala Ile Gly Tyr Phe Lys Lys Gln Gly Phe Ser Lys
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            660                 665                 670
Leu Ile Glu Arg Lys Gln Ala Gln Ile Arg Lys Val Tyr Pro Gly Leu
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705                 710                 715                 720
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Gln Gln Val Lys Ser His Gln Ser Ala Trp Pro Phe Met Glu Pro Val
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    770                 775                 780
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(2) INFORMATION FOR SEQ ID NO:2:

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(A) LENGTH: 481 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

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Asp Ile Pro Met Glu Leu Ile Asn Glu Val Met Ser Thr Ile Thr Asp
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Lys Thr Leu Ala Leu Ile Lys Asp Gly Arg Val Ile Gly Gly Ile Cys
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Val Thr Ser Asn Glu Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn
225                 230                 235                 240
His Leu Lys Glu Tyr His Ile Lys His Asp Ile Leu Asn Phe Leu Thr
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Lys Glu Ile Lys Ile Pro Lys Thr Lys Tyr Val Gly Tyr Ile Lys Asp
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Pro Gly Ile Arg Glu Thr Gly Trp Lys Pro Ser Gly Lys Glu Lys Ser
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Lys Glu Pro Arg Asp Pro Asp Gln Leu Tyr Ser Thr Leu Lys Ser Ile
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Val Lys Arg Thr Glu Ala Pro Gly Tyr Tyr Glu Val Ile Arg Ser Pro
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Met Asp Leu Lys Thr Met Ser Glu Arg Leu Lys Asn Arg Tyr Tyr Val
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(2) INFORMATION FOR SEQ ID NO:3:

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(A) LENGTH: 203 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

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        35                  40                  45
Gln Lys Leu Arg Gln Gln Gln Leu Gln His Arg Leu Gln Gln Ala Gln
    50                  55                  60
Met Leu Arg Arg Arg Met Ala Ser Met Arg Thr Gly Val Val Gly Gln
65                  70                  75                  80
Gln Gln Gly Leu Pro Ser Pro Thr Pro Ala Thr Pro Thr Thr Pro Thr
                85                  90                  95
Gly Gln Gln Pro Thr Thr Pro Gln Thr Pro Gln Pro Thr Ser Gln Pro
            100                 105                 110
Gln Pro Thr Pro Pro Asn Ser Met Pro Pro Tyr Leu Pro Arg Thr Gln
        115                 120                 125
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Pro Thr Pro Pro Gln Thr Ala Gln Pro Pro Leu Pro Gly Pro Pro Pro
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Thr Ala Val Glu Met Ala Met Gln Ile Gln Arg Ala Ala Glu Thr Gln
                165                 170                 175
Arg Gln Met Ala His Val Gln Ile Phe Gln Arg Pro Ile Gln His Gln
            180                 185                 190
Met Pro Pro Met Thr Pro Met Ala Pro Met Gly
        195                 200
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Glu Ala Gly Gly Ala Gly Pro Gly Gly Cys Gly Ala Gly Ala
 1               5                  10                  15
Gly Ala Gly Ala Gly Pro Gly Ala Leu Pro Pro Gln Pro Ala Ala Leu
            20                  25                  30
Pro Pro Ala Pro Pro Gln Gly Ser Pro Cys Ala Ala Ala Ala Gly Gly
        35                  40                  45
Ser Gly Ala Cys Gly Pro Ala Thr Ala Val Ala Ala Ala Gly Thr Ala
    50                  55                  60
Glu Gly Pro Gly Gly Gly Gly Ser Ala Arg Ile Ala Val Lys Lys Ala
65                  70                  75                  80
Gln Leu Arg Ser Ala Pro Arg Ala Lys Lys Leu Glu Lys Leu Gly Val
                85                  90                  95
Tyr Ser Ala Cys Lys Ala Glu Glu Ser Cys Lys Cys Asn Gly Trp Lys
            100                 105                 110
Asn Pro Asn Pro Ser Pro Thr Pro Pro Arg Ala Asp Leu Gln Gln Ile
        115                 120                 125
Ile Val Ser Leu Thr Glu Ser Cys Arg Ser Cys Ser His Ala Leu Ala
    130                 135                 140
```

```
Ala His Val Ser His Leu Glu Asn Val Ser Glu Glu Glu Met Asn Arg
145                 150                 155                 160
Leu Leu Gly Ile Val Leu Asp Val Glu Tyr Leu Phe Thr Cys Val His
                165                 170                 175
Lys Glu Glu Asp Ala Asp Thr Lys Gln Val Tyr Phe Tyr Leu Phe Lys
            180                 185                 190
Leu Leu Arg Lys Ser Ile Leu Gln Arg Gly Lys Pro Val Val Glu Gly
        195                 200                 205
Ser Leu Glu Lys Lys Pro Pro Phe Glu Lys Pro Ser Ile Glu Gln Gly
    210                 215                 220
Val Asn Asn Phe Val Gln Tyr Lys Phe Ser His Leu Pro Ala Lys Glu
225                 230                 235                 240
Arg Gln Thr Ile Val Glu Leu Ala Lys Met Phe Leu Asn Arg Ile Asn
                245                 250                 255
Tyr Trp His Leu Glu Ala Pro Ser Gln Arg Arg Leu Arg Ser Pro Asn
            260                 265                 270
Asp Asp Ile Ser Gly Tyr Lys Glu Asn Tyr Thr Arg Trp Leu Cys Tyr
        275                 280                 285
Cys Asn Val Pro Gln Phe Cys Asp Ser Leu Pro Arg Tyr Glu Thr Thr
    290                 295                 300
Gln Val Phe Gly Arg Thr Leu Leu Arg Ser Val Phe Thr Val Met Arg
305                 310                 315                 320
Arg Gln Leu Leu Glu Gln Ala Arg Gln Glu Lys Asp Lys Leu Pro Leu
                325                 330                 335
Glu Lys Arg Thr Leu Ile Leu Thr His Phe Pro Lys Phe Leu Ser
            340                 345                 350
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 476 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Leu Glu Glu Glu Ile Tyr Gly Ala Asn Ser Pro Ile Trp Glu Ser
 1                5                  10                  15
Gly Phe Thr Met Pro Pro Ser Glu Gly Thr Gln Leu Val Pro Arg Pro
            20                  25                  30
Ala Ser Val Ser Ala Ala Val Val Pro Ser Thr Pro Ile Phe Ser Pro
        35                  40                  45
Ser Met Gly Gly Gly Ser Asn Ser Ser Leu Ser Leu Asp Ser Ala Gly
    50                  55                  60
Ala Glu Pro Met Pro Gly Glu Lys Arg Thr Leu Pro Glu Asn Leu Thr
65                  70                  75                  80
Leu Glu Asp Ala Lys Arg Leu Arg Val Met Gly Asp Ile Pro Met Glu
                85                  90                  95
Leu Val Asn Glu Val Met Leu Thr Ile Thr Asp Pro Ala Ala Met Leu
            100                 105                 110
Gly Pro Glu Thr Ser Leu Leu Ser Ala Asn Ala Ala Arg Asp Glu Thr
        115                 120                 125
Ala Arg Leu Glu Glu Arg Arg Gly Ile Ile Glu Phe His Val Ile Gly
    130                 135                 140
Asn Ser Leu Thr Pro Lys Ala Asn Arg Arg Val Leu Leu Trp Leu Val
145                 150                 155                 160
Gly Leu Gln Asn Val Phe Ser His Gln Leu Pro Arg Met Pro Lys Glu
                165                 170                 175
Tyr Ile Ala Arg Leu Val Phe Asp Pro Lys His Lys Thr Leu Ala Leu
            180                 185                 190
```

```
Ile Lys Asp Gly Arg Val Ile Gly Gly Ile Cys Phe Arg Met Phe Pro
        195                 200                 205
Thr Gln Gly Phe Thr Glu Ile Val Phe Cys Ala Val Thr Ser Asn Glu
    210                 215                 220
Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn His Leu Lys Glu Tyr
225                 230                 235                 240
His Ile Lys His Asn Ile Leu Tyr Phe Leu Thr Tyr Ala Asp Glu Tyr
                245                 250                 255
Ala Ile Gly Tyr Phe Lys Lys Gln Gly Phe Ser Lys Asp Ile Lys Val
            260                 265                 270
Pro Lys Ser Arg Tyr Leu Gly Tyr Ile Lys Asp Tyr Glu Gly Ala Thr
        275                 280                 285
Leu Met Glu Cys Glu Leu Asn Pro Arg Ile Pro Tyr Thr Glu Leu Ser
    290                 295                 300
His Ile Ile Lys Lys Gln Lys Glu Ile Ile Lys Lys Leu Ile Glu Arg
305                 310                 315                 320
Lys Gln Ala Gln Ile Arg Lys Val Tyr Pro Gly Leu Ser Cys Phe Lys
                325                 330                 335
Glu Gly Val Arg Gln Ile Pro Val Glu Ser Val Pro Gly Ile Arg Glu
            340                 345                 350
Thr Gly Trp Lys Pro Leu Gly Lys Glu Lys Gly Lys Glu Leu Lys Asp
        355                 360                 365
Pro Asp Gln Leu Tyr Thr Thr Leu Lys Asn Leu Leu Ala Gln Ile Lys
    370                 375                 380
Ser His Pro Ser Ala Trp Pro Phe Met Glu Pro Val Lys Lys Ser Glu
385                 390                 395                 400
Ala Pro Asp Tyr Tyr Glu Val Ile Arg Phe Pro Ile Asp Leu Lys Thr
                405                 410                 415
Met Thr Glu Arg Leu Arg Ser Arg Tyr Tyr Val Thr Arg Lys Leu Phe
            420                 425                 430
Val Ala Asp Leu Gln Arg Val Ile Ala Asn Cys Arg Glu Tyr Asn Pro
        435                 440                 445
Pro Asp Ser Glu Tyr Cys Arg Cys Ala Ser Ala Leu Glu Lys Phe Phe
    450                 455                 460
Tyr Phe Lys Leu Lys Glu Gly Gly Leu Ile Asp Lys
465                 470                 475
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2414 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Glu Asn Val Val Glu Pro Gly Pro Pro Ser Ala Lys Arg Pro
 1               5                  10                  15
Lys Leu Ser Ser Pro Ala Leu Ser Ala Ser Ala Ser Asp Gly Thr Asp
            20                  25                  30
Phe Gly Ser Leu Phe Asp Leu Glu His Asp Leu Pro Asp Glu Leu Ile
        35                  40                  45
Asn Ser Thr Glu Leu Gly Leu Thr Asn Gly Gly Asp Ile Asn Gln Leu
    50                  55                  60
Gln Thr Ser Leu Gly Met Val Gln Asp Ala Ala Ser Lys His Lys Gln
65                  70                  75                  80
Leu Ser Glu Leu Leu Arg Ser Gly Ser Ser Pro Asn Leu Asn Met Gly
                85                  90                  95
Val Gly Gly Pro Gly Gln Val Met Ala Ser Gln Ala Gln Gln Ser Ser
            100                 105                 110
```

```
Pro Gly Leu Gly Leu Ile Asn Ser Met Val Lys Ser Pro Met Thr Gln
        115                 120                 125
Ala Gly Leu Thr Ser Pro Asn Met Gly Met Gly Thr Ser Gly Pro Asn
    130                 135                 140
Gln Gly Pro Thr Gln Ser Thr Gly Met Met Asn Ser Pro Val Asn Gln
145                 150                 155                 160
Pro Ala Met Gly Met Asn Thr Gly Thr Asn Ala Gly Met Asn Pro Gly
                165                 170                 175
Met Leu Ala Ala Gly Asn Gly Gln Gly Ile Met Pro Asn Gln Val Met
            180                 185                 190
Asn Gly Ser Ile Gly Ala Gly Arg Gly Arg Gln Asp Met Gln Tyr Pro
        195                 200                 205
Asn Pro Gly Met Gly Ser Ala Gly Asn Leu Leu Thr Glu Pro Leu Gln
    210                 215                 220
Gln Gly Ser Pro Gln Met Gly Gly Gln Thr Gly Leu Arg Gly Pro Gln
225                 230                 235                 240
Pro Leu Lys Met Gly Met Met Asn Asn Pro Asn Pro Tyr Gly Ser Pro
                245                 250                 255
Tyr Thr Gln Asn Pro Gly Gln Gln Ile Gly Ala Ser Gly Leu Gly Leu
            260                 265                 270
Gln Ile Gln Thr Lys Thr Val Leu Ser Asn Asn Leu Ser Pro Phe Ala
        275                 280                 285
Met Asp Lys Lys Ala Val Pro Gly Gly Gly Met Pro Asn Met Gly Gln
    290                 295                 300
Gln Pro Ala Pro Gln Val Gln Gln Pro Gly Leu Val Thr Pro Val Ala
305                 310                 315                 320
Gln Gly Met Gly Ser Gly Ala His Thr Ala Asp Pro Glu Lys Arg Lys
                325                 330                 335
Leu Ile Gln Gln Gln Leu Val Leu Leu Leu His Ala His Lys Cys Gln
            340                 345                 350
Arg Arg Glu Gln Ala Asn Gly Glu Val Arg Gln Cys Asn Leu Pro His
        355                 360                 365
Cys Arg Thr Met Lys Asn Val Leu Asn His Met Thr His Cys Gln Ser
    370                 375                 380
Gly Lys Ser Cys Gln Val Ala His Cys Ala Ser Ser Arg Gln Ile Ile
385                 390                 395                 400
Ser His Trp Lys Asn Cys Thr Arg His Asp Cys Pro Val Cys Leu Pro
                405                 410                 415
Leu Lys Asn Ala Gly Asp Lys Arg Asn Gln Gln Pro Ile Leu Thr Gly
            420                 425                 430
Ala Pro Val Gly Leu Gly Asn Pro Ser Ser Leu Gly Val Gly Gln Gln
        435                 440                 445
Ser Ala Pro Asn Leu Ser Thr Val Ser Gln Ile Asp Pro Ser Ser Ile
    450                 455                 460
Glu Arg Ala Tyr Ala Ala Leu Gly Leu Pro Tyr Gln Val Asn Gln Met
465                 470                 475                 480
Pro Thr Gln Pro Gln Val Gln Ala Lys Asn Gln Gln Asn Gln Gln Pro
                485                 490                 495
Gly Gln Ser Pro Gln Gly Met Arg Pro Met Ser Asn Met Ser Ala Ser
            500                 505                 510
Pro Met Gly Val Asn Gly Gly Val Gly Val Gln Thr Pro Ser Leu Leu
        515                 520                 525
Ser Asp Ser Met Leu His Ser Ala Ile Asn Ser Gln Asn Pro Met Met
    530                 535                 540
Ser Glu Asn Ala Ser Val Pro Ser Leu Gly Pro Met Pro Thr Ala Ala
545                 550                 555                 560
Gln Pro Ser Thr Thr Gly Ile Arg Lys Gln Trp His Glu Asp Ile Thr
                565                 570                 575
Gln Asp Leu Arg Asn His Leu Val His Lys Leu Val Gln Ala Ile Phe
            580                 585                 590
Pro Thr Pro Asp Pro Ala Ala Leu Lys Asp Arg Arg Met Glu Asn Leu
        595                 600                 605
```

```
Val Ala Tyr Ala Arg Lys Val Glu Gly Asp Met Tyr Glu Ser Ala Asn
    610                 615                 620
Asn Arg Ala Glu Tyr Tyr His Leu Leu Ala Glu Lys Ile Tyr Lys Ile
625                 630                 635                 640
Gln Lys Glu Leu Glu Glu Lys Arg Arg Thr Arg Leu Gln Lys Gln Asn
                645                 650                 655
Met Leu Pro Asn Ala Ala Gly Met Val Pro Val Ser Met Asn Pro Gly
            660                 665                 670
Pro Asn Met Gly Gln Pro Gln Pro Gly Met Thr Ser Asn Gly Pro Leu
        675                 680                 685
Pro Asp Pro Ser Met Ile Arg Gly Ser Val Pro Asn Gln Met Met Pro
    690                 695                 700
Arg Ile Thr Pro Gln Ser Gly Leu Asn Gln Phe Gly Gln Met Ser Met
705                 710                 715                 720
Ala Gln Pro Pro Ile Val Pro Arg Gln Thr Pro Pro Leu Gln His His
                725                 730                 735
Gly Gln Leu Ala Gln Pro Gly Ala Leu Asn Pro Pro Met Gly Tyr Gly
            740                 745                 750
Pro Arg Met Gln Gln Pro Ser Asn Gln Gly Gln Phe Leu Pro Gln Thr
        755                 760                 765
Gln Phe Pro Ser Gln Gly Met Asn Val Thr Asn Ile Pro Leu Ala Pro
    770                 775                 780
Ser Ser Gly Gln Ala Pro Val Ser Gln Ala Gln Met Ser Ser Ser Ser
785                 790                 795                 800
Cys Pro Val Asn Ser Pro Ile Met Pro Pro Gly Ser Gln Gly Ser His
                805                 810                 815
Ile His Cys Pro Gln Leu Pro Gln Pro Ala Leu His Gln Asn Ser Pro
            820                 825                 830
Ser Pro Val Pro Ser Arg Thr Pro Thr Pro His His Thr Pro Pro Ser
        835                 840                 845
Ile Gly Ala Gln Gln Pro Pro Ala Thr Thr Ile Pro Ala Pro Val Pro
    850                 855                 860
Thr Pro Pro Ala Met Pro Pro Gly Pro Gln Ser Gln Ala Leu His Pro
865                 870                 875                 880
Pro Pro Arg Gln Thr Pro Thr Pro Pro Thr Thr Gln Leu Pro Gln Gln
                885                 890                 895
Val Gln Pro Ser Leu Pro Ala Ala Pro Ser Ala Asp Gln Pro Gln Gln
            900                 905                 910
Gln Pro Arg Ser Gln Gln Ser Thr Ala Ala Ser Val Pro Thr Pro Asn
        915                 920                 925
Ala Pro Leu Leu Pro Pro Gln Pro Ala Thr Pro Leu Ser Gln Pro Ala
    930                 935                 940
Val Ser Ile Glu Gly Gln Val Ser Asn Pro Pro Ser Thr Ser Ser Thr
945                 950                 955                 960
Glu Val Asn Ser Gln Ala Ile Ala Glu Lys Gln Pro Ser Gln Glu Val
                965                 970                 975
Lys Met Glu Ala Lys Met Glu Val Asp Gln Pro Glu Pro Ala Asp Thr
            980                 985                 990
Gln Pro Glu Asp Ile Ser Glu Ser Lys Val Glu Asp Cys Lys Met Glu
        995                 1000                1005
Ser Thr Glu Thr Glu Glu Arg Ser Thr Glu Leu Lys Thr Glu Ile Lys
    1010                1015                1020
Glu Glu Glu Asp Gln Pro Ser Thr Ser Ala Thr Gln Ser Ser Pro Ala
025                 1030                1035                1040
Pro Gly Gln Ser Lys Lys Lys Ile Phe Lys Pro Glu Glu Leu Arg Gln
                1045                1050                1055
Ala Leu Met Pro Thr Leu Glu Ala Leu Tyr Arg Gln Asp Pro Glu Ser
            1060                1065                1070
Leu Pro Phe Arg Gln Pro Val Asp Pro Gln Leu Leu Gly Ile Pro Asp
        1075                1080                1085
Tyr Phe Asp Ile Val Lys Ser Pro Met Asp Leu Ser Thr Ile Lys Arg
    1090                1095                1100
```

```
Lys Leu Asp Thr Gly Gln Tyr Gln Glu Pro Trp Gln Tyr Val Asp Asp
105                 1110                1115                1120
Ile Trp Leu Met Phe Asn Asn Ala Trp Leu Tyr Asn Arg Lys Thr Ser
                1125                1130                1135
Arg Val Tyr Lys Tyr Cys Ser Lys Leu Ser Glu Val Phe Glu Gln Glu
            1140                1145                1150
Ile Asp Pro Val Met Gln Ser Leu Gly Tyr Cys Cys Gly Arg Lys Leu
        1155                1160                1165
Glu Phe Ser Pro Gln Thr Leu Cys Cys Tyr Gly Lys Gln Leu Cys Thr
    1170                1175                1180
Ile Pro Arg Asp Ala Thr Tyr Tyr Ser Tyr Gln Asn Arg Tyr His Phe
185                 1190                1195                1200
Cys Glu Lys Cys Phe Asn Glu Ile Gln Gly Glu Ser Val Ser Leu Gly
                1205                1210                1215
Asp Asp Pro Ser Gln Pro Gln Thr Thr Ile Asn Lys Glu Gln Phe Ser
            1220                1225                1230
Lys Arg Lys Asn Asp Thr Leu Asp Pro Glu Leu Phe Val Glu Cys Thr
        1235                1240                1245
Glu Cys Gly Arg Lys Met His Gln Ile Cys Val Leu His His Glu Ile
    1250                1255                1260
Ile Trp Pro Ala Gly Phe Val Cys Asp Gly Cys Leu Lys Lys Ser Ala
265                 1270                1275                1280
Arg Thr Arg Lys Glu Asn Lys Phe Ser Ala Lys Arg Leu Pro Ser Thr
                1285                1290                1295
Arg Leu Gly Thr Phe Leu Glu Asn Arg Val Asn Asp Phe Leu Arg Arg
            1300                1305                1310
Gln Asn His Pro Glu Ser Gly Glu Val Thr Val Arg Val Val His Ala
        1315                1320                1325
Ser Asp Lys Thr Val Glu Val Lys Pro Gly Met Lys Ala Arg Phe Val
    1330                1335                1340
Asp Ser Gly Glu Met Ala Glu Ser Phe Pro Tyr Arg Thr Lys Ala Leu
345                 1350                1355                1360
Phe Ala Phe Glu Glu Ile Asp Gly Val Asp Leu Cys Phe Phe Gly Met
                1365                1370                1375
His Val Gln Glu Tyr Gly Ser Asp Cys Pro Pro Pro Asn Gln Arg Arg
            1380                1385                1390
Val Tyr Ile Ser Tyr Leu Asp Ser Val His Phe Phe Arg Pro Lys Cys
        1395                1400                1405
Leu Arg Thr Ala Val Tyr His Glu Ile Leu Ile Gly Tyr Leu Glu Tyr
    1410                1415                1420
Val Lys Lys Leu Gly Tyr Thr Thr Gly His Ile Trp Ala Cys Pro Pro
425                 1430                1435                1440
Ser Glu Gly Asp Asp Tyr Ile Phe His Cys His Pro Pro Asp Gln Lys
                1445                1450                1455
Ile Pro Lys Pro Lys Arg Leu Gln Glu Trp Tyr Lys Lys Met Leu Asp
            1460                1465                1470
Lys Ala Val Ser Glu Arg Ile Val His Asp Tyr Lys Asp Ile Phe Lys
        1475                1480                1485
Gln Ala Thr Glu Asp Arg Leu Thr Ser Ala Lys Glu Leu Pro Tyr Phe
    1490                1495                1500
Glu Gly Asp Phe Trp Pro Asn Val Leu Glu Glu Ser Ile Lys Glu Leu
505                 1510                1515                1520
Glu Gln Glu Glu Glu Glu Arg Lys Arg Glu Glu Asn Thr Ser Asn Glu
                1525                1530                1535
Ser Thr Asp Val Thr Lys Gly Asp Ser Lys Asn Ala Lys Lys Lys Asn
            1540                1545                1550
Asn Lys Lys Thr Ser Lys Asn Lys Ser Ser Leu Ser Arg Gly Asn Lys
        1555                1560                1565
Lys Lys Pro Gly Met Pro Asn Val Ser Asn Asp Leu Ser Gln Lys Leu
    1570                1575                1580
Tyr Ala Thr Met Glu Lys His Lys Glu Val Phe Phe Val Ile Arg Leu
585                 1590                1595                1600
```

Ile Ala Gly Pro Ala Ala Asn Ser Leu Pro Pro Ile Val Asp Pro Asp
1605 1610 1615
Pro Leu Ile Pro Cys Asp Leu Met Asp Gly Arg Asp Ala Phe Leu Thr
1620 1625 1630
Leu Ala Arg Asp Lys His Leu Glu Phe Ser Ser Leu Arg Arg Ala Gln
1635 1640 1645
Trp Ser Thr Met Cys Met Leu Val Glu Leu His Thr Gln Ser Gln Asp
1650 1655 1660
Arg Phe Val Tyr Thr Cys Asn Glu Cys Lys His His Val Glu Thr Arg
665 1670 1675 1680
Trp His Cys Thr Val Cys Glu Asp Tyr Asp Leu Cys Ile Thr Cys Tyr
1685 1690 1695
Asn Thr Lys Asn His Asp His Lys Met Glu Lys Leu Gly Leu Gly Leu
1700 1705 1710
Asp Asp Glu Ser Asn Asn Gln Gln Ala Ala Ala Thr Gln Ser Pro Gly
1715 1720 1725
Asp Ser Arg Arg Leu Ser Ile Gln Arg Cys Ile Gln Ser Leu Val His
1730 1735 1740
Ala Cys Gln Cys Arg Asn Ala Asn Cys Ser Leu Pro Ser Cys Gln Lys
745 1750 1755 1760
Met Lys Arg Val Val Gln His Thr Lys Gly Cys Lys Arg Lys Thr Asn
1765 1770 1775
Gly Gly Cys Pro Ile Cys Lys Gln Leu Ile Ala Leu Cys Cys Tyr His
1780 1785 1790
Ala Lys His Cys Gln Glu Asn Lys Cys Pro Val Pro Phe Cys Leu Asn
1795 1800 1805
Ile Lys Gln Lys Leu Arg Gln Gln Gln Leu Gln His Arg Leu Gln Gln
1810 1815 1820
Ala Gln Met Leu Arg Arg Arg Met Ala Ser Met Gln Arg Thr Gly Val
825 1830 1835 1840
Val Gly Gln Gln Gln Gly Leu Pro Ser Pro Thr Pro Ala Thr Pro Thr
1845 1850 1855
Thr Pro Thr Gly Gln Gln Pro Thr Thr Pro Gln Thr Pro Gln Pro Thr
1860 1865 1870
Ser Gln Pro Gln Pro Thr Pro Pro Asn Ser Met Pro Pro Tyr Leu Pro
1875 1880 1885
Arg Thr Gln Ala Ala Gly Pro Val Ser Gln Gly Lys Ala Ala Gly Gln
1890 1895 1900
Val Thr Pro Pro Thr Pro Pro Gln Thr Ala Gln Pro Pro Leu Pro Gly
905 1910 1915 1920
Pro Pro Pro Thr Ala Val Glu Met Ala Met Gln Ile Gln Arg Ala Ala
1925 1930 1935
Glu Thr Gln Arg Gln Met Ala His Val Gln Ile Phe Gln Arg Pro Ile
1940 1945 1950
Gln His Gln Met Pro Pro Met Thr Pro Met Ala Pro Met Gly Met Asn
1955 1960 1965
Pro Pro Pro Met Thr Arg Gly Pro Ser Gly His Leu Glu Pro Gly Met
1970 1975 1980
Gly Pro Thr Gly Met Gln Gln Gln Pro Pro Trp Ser Gln Gly Gly Leu
985 1990 1995 2000
Pro Gln Pro Gln Gln Leu Gln Ser Gly Met Pro Arg Pro Ala Met Met
2005 2010 2015
Ser Val Ala Gln His Gly Gln Pro Leu Asn Met Ala Pro Gln Pro Gly
2020 2025 2030
Leu Gly Gln Val Gly Ile Ser Pro Leu Lys Pro Gly Thr Val Ser Gln
2035 2040 2045
Gln Ala Leu Gln Asn Leu Leu Arg Thr Leu Arg Ser Pro Ser Ser Pro
2050 2055 2060
Leu Gln Gln Gln Gln Val Leu Ser Ile Leu His Ala Asn Pro Gln Leu
065 2070 2075 2080
Leu Ala Ala Phe Ile Lys Gln Arg Ala Ala Lys Tyr Ala Asn Ser Asn
2085 2090 2095

```
Pro Gln Pro Ile Pro Gly Gln Pro Gly Met Pro Gln Gly Gln Pro Gly
          2100                2105                2110
Leu Gln Pro Pro Thr Met Pro Gly Gln Gln Gly Val His Ser Asn Pro
      2115                2120                2125
Ala Met Gln Asn Met Asn Pro Met Gln Ala Gly Val Gln Arg Ala Gly
  2130                2135                2140
Leu Pro Gln Gln Gln Pro Gln Gln Gln Leu Gln Pro Pro Met Gly Gly
145                 2150                2155                2160
Met Ser Pro Gln Ala Gln Gln Met Asn Met Asn His Asn Thr Met Pro
                2165                2170                2175
Ser Gln Phe Arg Asp Ile Leu Arg Arg Gln Gln Met Met Gln Gln Gln
          2180                2185                2190
Gln Gln Gln Gly Ala Gly Pro Gly Ile Gly Pro Gly Met Ala Asn His
      2195                2200                2205
Asn Gln Phe Gln Gln Pro Gln Gly Val Gly Tyr Pro Pro Gln Pro Gln
  2210                2215                2220
Gln Arg Met Gln His His Met Gln Gln Met Gln Gln Gly Asn Met Gly
225                 2230                2235                2240
Gln Ile Gly Gln Leu Pro Gln Ala Leu Gly Ala Glu Ala Gly Ala Ser
                2245                2250                2255
Leu Gln Ala Tyr Gln Gln Arg Leu Leu Gln Gln Gln Met Gly Ser Pro
          2260                2265                2270
Val Gln Pro Asn Pro Met Ser Pro Gln Gln His Met Leu Pro Asn Gln
      2275                2280                2285
Ala Gln Ser Pro His Leu Gln Gly Gln Gln Ile Pro Asn Ser Leu Ser
  2290                2295                2300
Asn Gln Val Arg Ser Pro Gln Pro Val Pro Ser Pro Arg Pro Gln Ser
305                 2310                2315                2320
Gln Pro Pro His Ser Ser Pro Ser Pro Arg Met Gln Pro Gln Pro Ser
                2325                2330                2335
Pro His His Val Ser Pro Gln Thr Ser Ser Pro His Pro Gly Leu Val
          2340                2345                2350
Ala Ala Gln Ala Asn Pro Met Glu Gln Gly His Phe Ala Ser Pro Asp
      2355                2360                2365
Gln Asn Ser Met Leu Ser Gln Leu Ala Ser Asn Pro Gly Met Ala Asn
  2370                2375                2380
Leu His Gly Ala Ser Ala Thr Asp Leu Gly Leu Ser Thr Asp Asn Ser
385                 2390                2395                2400
Asp Leu Asn Ser Asn Leu Ser Gln Ser Thr Leu Asp Ile His
                2405                2410                2
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2441 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Ala Glu Asn Leu Leu Asp Gly Pro Pro Asn Pro Lys Arg Ala Lys
 1               5                  10                  15
Leu Ser Ser Pro Gly Phe Ser Ala Asn Asp Asn Thr Asp Phe Gly Ser
            20                  25                  30
Leu Phe Asp Leu Glu Asn Asp Leu Pro Asp Glu Leu Ile Pro Asn Gly
        35                  40                  45
Glu Leu Ser Leu Leu Asn Ser Gly Asn Leu Val Pro Asp Ala Ala Ser
    50                  55                  60
Lys His Lys Gln Leu Ser Glu Leu Leu Arg Gly Gly Ser Gly Ser Ser
65                  70                  75                  80
```

```
Ile Asn Pro Gly Ile Gly Asn Val Ser Ala Ser Ser Pro Val Gln Gln
                85                  90                  95
Gly Leu Gly Gly Gln Ala Gln Gly Gln Pro Asn Ser Thr Asn Met Ala
            100                 105                 110
Ser Leu Gly Ala Met Gly Lys Ser Pro Leu Asn Gln Gly Asp Ser Ser
        115                 120                 125
Thr Pro Asn Leu Pro Lys Gln Ala Ala Ser Thr Ser Gly Pro Thr Pro
    130                 135                 140
Pro Ala Ser Gln Ala Leu Asn Pro Gln Ala Gln Lys Gln Val Gly Leu
145                 150                 155                 160
Val Thr Ser Ser Pro Ala Thr Ser Gln Thr Gly Pro Gly Ile Cys Met
                165                 170                 175
Asn Ala Asn Phe Asn Gln Thr His Pro Gly Leu Leu Asn Ser Asn Ser
            180                 185                 190
Gly His Ser Leu Met Asn Gln Ala Gln Gln Gly Gln Ala Gln Val Met
        195                 200                 205
Asn Gly Ser Leu Gly Ala Ala Gly Arg Gly Arg Gly Ala Gly Met Pro
    210                 215                 220
Tyr Pro Ala Pro Ala Met Gln Gly Ala Thr Ser Ser Val Leu Ala Glu
225                 230                 235                 240
Thr Leu Thr Gln Val Ser Pro Gln Met Ala Gly His Ala Gly Leu Asn
                245                 250                 255
Thr Ala Gln Ala Gly Gly Met Thr Lys Met Gly Met Thr Gly Thr Thr
            260                 265                 270
Ser Pro Phe Gly Gln Pro Phe Ser Gln Thr Gly Gly Gln Gln Met Gly
        275                 280                 285
Ala Thr Gly Val Asn Pro Gln Leu Ala Ser Lys Gln Ser Met Val Asn
    290                 295                 300
Ser Leu Pro Ala Phe Pro Thr Asp Ile Lys Asn Thr Ser Val Thr Thr
305                 310                 315                 320
Val Pro Asn Met Ser Gln Leu Gln Thr Ser Val Gly Ile Val Pro Thr
                325                 330                 335
Gln Ala Ile Ala Thr Gly Pro Thr Ala Asp Pro Glu Lys Arg Lys Leu
            340                 345                 350
Ile Gln Gln Gln Leu Val Leu Leu Leu His Ala His Lys Cys Gln Arg
        355                 360                 365
Arg Glu Gln Ala Asn Gly Glu Val Arg Ala Cys Ser Leu Pro His Cys
    370                 375                 380
Arg Thr Met Lys Asn Val Leu Asn His Met Thr His Cys Gln Ala Pro
385                 390                 395                 400
Lys Ala Cys Gln Val Ala His Cys Ala Ser Ser Arg Gln Ile Ile Ser
                405                 410                 415
His Trp Lys Asn Cys Thr Arg His Asp Cys Pro Val Cys Leu Pro Leu
            420                 425                 430
Lys Asn Ala Ser Asp Lys Arg Asn Gln Gln Thr Ile Leu Gly Ser Pro
        435                 440                 445
Ala Ser Gly Ile Gln Asn Thr Ile Gly Ser Val Gly Ala Gly Gln Gln
    450                 455                 460
Asn Ala Thr Ser Leu Ser Asn Pro Asn Pro Ile Asp Pro Ser Ser Met
465                 470                 475                 480
Gln Arg Ala Tyr Ala Ala Leu Gly Leu Pro Tyr Met Asn Gln Pro Gln
                485                 490                 495
Thr Gln Leu Gln Pro Gln Val Pro Gly Gln Gln Pro Ala Gln Pro Pro
            500                 505                 510
Ala His Gln Gln Met Arg Thr Leu Asn Ala Leu Gly Asn Asn Pro Met
        515                 520                 525
Ser Val Pro Ala Gly Gly Ile Thr Thr Asp Gln Gln Pro Pro Asn Leu
    530                 535                 540
Ile Ser Glu Ser Ala Leu Pro Thr Ser Leu Gly Ala Thr Asn Pro Leu
545                 550                 555                 560
Met Asn Asp Gly Ser Asn Ser Gly Asn Ile Gly Ser Leu Ser Thr Ile
                565                 570                 575
```

```
Pro Thr Ala Ala Pro Pro Ser Ser Thr Gly Val Arg Lys Gly Trp His
            580                 585                 590
Glu His Val Thr Gln Asp Leu Arg Ser His Leu Val His Lys Leu Val
        595                 600                 605
Gln Ala Ile Phe Pro Thr Pro Asp Pro Ala Ala Leu Lys Asp Arg Arg
    610                 615                 620
Met Glu Asn Leu Val Ala Tyr Ala Lys Lys Val Glu Gly Asp Met Tyr
625                 630                 635                 640
Glu Ser Ala Asn Ser Arg Asp Glu Tyr Tyr His Leu Leu Ala Glu Lys
                645                 650                 655
Ile Tyr Lys Ile Gln Lys Glu Leu Glu Glu Lys Arg Arg Thr Arg Leu
            660                 665                 670
His Lys Gln Gly Ile Leu Gly Asn Gln Pro Ala Leu Pro Ala Ser Gly
        675                 680                 685
Ala Gln Pro Pro Val Ile Pro Pro Ala Gln Ser Val Arg Pro Pro Asn
    690                 695                 700
Gly Pro Leu Pro Leu Pro Val Asn Arg Met Gln Val Ser Gln Gly Met
705                 710                 715                 720
Asn Ser Phe Asn Pro Met Ser Leu Gly Asn Val Gln Leu Pro Gln Ala
                725                 730                 735
Pro Met Gly Pro Arg Ala Ala Ser Pro Met Asn His Ser Val Gln Met
            740                 745                 750
Asn Ser Met Ala Ser Val Pro Gly Met Ala Ile Ser Pro Ser Arg Met
        755                 760                 765
Pro Gln Pro Pro Asn Met Met Gly Thr His Ala Asn Asn Ile Met Ala
    770                 775                 780
Gln Ala Pro Thr Gln Asn Gln Phe Leu Pro Gln Asn Gln Phe Pro Ser
785                 790                 795                 800
Ser Ser Gly Ala Met Ser Val Asn Ser Val Gly Met Gly Gln Pro Ala
                805                 810                 815
Ala Gln Ala Gly Val Ser Gln Gly Gln Glu Pro Gly Ala Ala Leu Pro
            820                 825                 830
Asn Pro Leu Asn Met Leu Ala Pro Gln Ala Ser Gln Leu Pro Cys Pro
        835                 840                 845
Pro Val Thr Gln Ser Pro Leu His Pro Thr Pro Pro Pro Ala Ser Thr
    850                 855                 860
Ala Ala Gly Met Pro Ser Leu Gln His Pro Thr Ala Pro Gly Met Thr
865                 870                 875                 880
Pro Pro Gln Pro Ala Ala Pro Thr Gln Pro Ser Thr Pro Val Ser Ser
                885                 890                 895
Gly Gln Thr Pro Thr Pro Thr Pro Gly Ser Val Pro Ser Ala Ala Gln
            900                 905                 910
Thr Gln Ser Thr Pro Thr Val Gln Ala Ala Ala Gln Ala Gln Val Thr
        915                 920                 925
Pro Gln Pro Gln Thr Pro Val Gln Pro Pro Ser Val Ala Thr Pro Gln
    930                 935                 940
Ser Ser Gln Gln Gln Pro Thr Pro Val His Thr Gln Pro Pro Gly Thr
945                 950                 955                 960
Pro Leu Ser Gln Ala Ala Ala Ser Ile Asp Asn Arg Val Pro Thr Pro
                965                 970                 975
Ser Thr Val Thr Ser Ala Glu Thr Ser Ser Gln Gln Pro Gly Pro Asp
            980                 985                 990
Val Pro Met Leu Glu Met Lys Thr Glu Val Gln Thr Asp Asp Ala Glu
        995                1000                1005
Pro Glu Pro Thr Glu Ser Lys Gly Glu Pro Arg Ser Glu Met Met Glu
   1010                1015                1020
Glu Asp Leu Gln Gly Ser Ser Gln Val Lys Glu Glu Thr Asp Thr Thr
025                1030                1035                1040
Glu Gln Lys Ser Glu Pro Met Glu Val Glu Glu Lys Lys Pro Glu Val
               1045                1050                1055
Lys Val Glu Ala Lys Glu Glu Glu Glu Asn Ser Ser Asn Asp Thr Ala
           1060                1065                1070
```

Ser Gln Ser Thr Ser Pro Ser Gln Pro Arg Lys Lys Ile Phe Lys Pro
1075 1080 1085
Glu Glu Leu Arg Gln Ala Leu Met Pro Thr Leu Glu Ala Leu Tyr Arg
1090 1095 1100
Gln Asp Pro Glu Ser Leu Pro Phe Arg Gln Pro Val Asp Pro Gln Leu
105 1110 1115 1120
Leu Gly Ile Pro Asp Tyr Phe Asp Ile Val Lys Asn Pro Met Asp Leu
1125 1130 1135
Ser Thr Ile Lys Arg Lys Leu Asp Thr Gly Gln Tyr Gln Glu Pro Trp
1140 1145 1150
Gln Tyr Val Asp Asp Val Arg Leu Met Phe Asn Asn Ala Trp Leu Tyr
1155 1160 1165
Asn Arg Lys Thr Ser Arg Val Tyr Lys Phe Cys Ser Lys Leu Ala Glu
1170 1175 1180
Val Phe Glu Gln Glu Ile Asp Pro Val Met Gln Ser Leu Gly Tyr Cys
185 1190 1195 1200
Cys Gly Arg Lys Tyr Glu Phe Ser Pro Gln Thr Leu Cys Cys Tyr Gly
1205 1210 1215
Lys Gln Leu Cys Thr Ile Pro Arg Asp Ala Ala Tyr Tyr Ser Tyr Gln
1220 1225 1230
Asn Arg Tyr His Phe Cys Gly Lys Cys Phe Thr Glu Ile Gln Gly Glu
1235 1240 1245
Asn Val Thr Leu Gly Asp Asp Pro Ser Gln Pro Gln Thr Thr Ile Ser
1250 1255 1260
Lys Asp Gln Phe Glu Lys Lys Lys Asn Asp Thr Leu Asp Pro Glu Pro
265 1270 1275 1280
Phe Val Asp Cys Lys Glu Cys Gly Arg Lys Met His Gln Ile Cys Val
1285 1290 1295
Leu His Tyr Asp Ile Ile Trp Pro Ser Gly Phe Val Cys Asp Asn Cys
1300 1305 1310
Leu Lys Lys Thr Gly Arg Pro Arg Lys Glu Asn Lys Phe Ser Ala Lys
1315 1320 1325
Arg Leu Gln Thr Thr Arg Leu Gly Asn His Leu Glu Asp Arg Val Asn
1330 1335 1340
Lys Phe Leu Arg Arg Gln Asn His Pro Glu Ala Gly Glu Val Phe Val
345 1350 1355 1360
Arg Val Val Ala Ser Ser Asp Lys Thr Val Glu Val Lys Pro Gly Met
1365 1370 1375
Lys Ser Arg Phe Val Asp Ser Gly Glu Met Ser Glu Ser Phe Pro Tyr
1380 1385 1390
Arg Thr Lys Ala Leu Phe Ala Phe Glu Glu Ile Asp Gly Val Asp Val
1395 1400 1405
Cys Phe Phe Gly Met His Val Gln Asp Thr Ala Leu Ile Ala Pro His
1410 1415 1420
Gln Ile Gln Gly Cys Val Tyr Ile Ser Tyr Leu Asp Ser Ile His Phe
425 1430 1435 1440
Phe Arg Pro Arg Cys Leu Arg Thr Ala Val Tyr His Glu Ile Leu Ile
1445 1450 1455
Gly Tyr Leu Glu Tyr Val Lys Lys Leu Val Tyr Val Thr Ala His Ile
1460 1465 1470
Trp Ala Cys Pro Pro Ser Glu Gly Asp Asp Tyr Ile Phe His Cys His
1475 1480 1485
Pro Pro Asp Gln Lys Ile Pro Lys Pro Lys Arg Leu Gln Glu Trp Tyr
1490 1495 1500
Lys Lys Met Leu Asp Lys Ala Phe Ala Glu Arg Ile Ile Asn Asp Tyr
505 1510 1515 1520
Lys Asp Ile Phe Lys Gln Ala Asn Glu Asp Arg Leu Thr Ser Ala Lys
1525 1530 1535
Glu Leu Pro Tyr Phe Glu Gly Asp Phe Trp Pro Asn Val Leu Glu Glu
1540 1545 1550
Ser Ile Lys Glu Leu Glu Gln Glu Glu Glu Glu Arg Lys Lys Glu Glu
1555 1560 1565

Ser Thr Ala Ala Ser Glu Thr Pro Glu Gly Ser Gln Gly Asp Ser Lys
1570 1575 1580
Asn Ala Lys Lys Lys Asn Asn Lys Lys Thr Asn Lys Asn Lys Ser Ser
585 1590 1595 1600
Ile Ser Arg Ala Asn Lys Lys Lys Pro Ser Met Pro Asn Val Ser Asn
1605 1610 1615
Asp Leu Ser Gln Lys Leu Tyr Ala Thr Met Glu Lys His Lys Glu Val
1620 1625 1630
Phe Phe Val Ile His Leu His Ala Gly Pro Val Ile Ser Thr Gln Pro
1635 1640 1645
Pro Ile Val Asp Pro Asp Pro Leu Leu Ser Cys Asp Leu Met Asp Gly
1650 1655 1660
Arg Asp Ala Phe Leu Thr Leu Ala Arg Asp Lys His Trp Glu Phe Ser
665 1670 1675 1680
Ser Leu Arg Arg Ser Lys Trp Ser Thr Leu Cys Met Leu Val Glu Leu
1685 1690 1695
His Thr Gln Gly Gln Asp Arg Phe Val Tyr Thr Cys Asn Glu Cys Lys
1700 1705 1710
His His Val Glu Thr Arg Trp His Cys Thr Val Cys Glu Asp Tyr Asp
1715 1720 1725
Leu Cys Ile Asn Cys Tyr Asn Thr Lys Ser His Thr His Lys Met Val
1730 1735 1740
Lys Trp Gly Leu Gly Leu Asp Asp Glu Gly Ser Ser Gln Gly Glu Pro
745 1750 1755 1760
Gln Ser Lys Ser Pro Gln Glu Ser Arg Arg Leu Ser Ile Gln Arg Cys
1765 1770 1775
Ile Gln Ser Leu Val His Ala Cys Gln Cys Arg Asn Ala Asn Cys Ser
1780 1785 1790
Leu Pro Ser Cys Gln Lys Met Lys Arg Val Val Gln His Thr Lys Gly
1795 1800 1805
Cys Lys Arg Lys Thr Asn Gly Gly Cys Pro Val Cys Lys Gln Leu Ile
1810 1815 1820
Ala Leu Cys Cys Tyr His Ala Lys His Cys Gln Glu Asn Lys Cys Pro
825 1830 1835 1840
Val Pro Phe Cys Leu Asn Ile Lys His Asn Val Arg Gln Gln Gln Ile
1845 1850 1855
Gln His Cys Leu Gln Gln Ala Gln Leu Met Arg Arg Arg Met Ala Thr
1860 1865 1870
Met Asn Thr Arg Asn Val Pro Gln Gln Ser Leu Pro Ser Pro Thr Ser
1875 1880 1885
Ala Pro Pro Gly Thr Pro Thr Gln Gln Pro Ser Thr Pro Gln Thr Pro
1890 1895 1900
Gln Pro Pro Ala Gln Pro Gln Pro Ser Pro Val Asn Met Ser Pro Ala
905 1910 1915 1920
Gly Phe Pro Asn Val Ala Arg Thr Gln Pro Pro Thr Ile Val Ser Ala
1925 1930 1935
Gly Lys Pro Thr Asn Gln Val Pro Ala Pro Pro Pro Pro Ala Gln Pro
1940 1945 1950
Pro Pro Ala Ala Val Glu Ala Ala Arg Gln Ile Glu Arg Glu Ala Gln
1955 1960 1965
Gln Gln Gln His Leu Tyr Arg Ala Asn Ile Asn Asn Gly Met Pro Pro
1970 1975 1980
Gly Arg Asp Gly Met Gly Thr Pro Gly Ser Gln Met Thr Pro Val Gly
985 1990 1995 2000
Leu Asn Val Pro Arg Pro Asn Gln Val Ser Gly Pro Val Met Ser Ser
2005 2010 2015
Met Pro Pro Gly Gln Trp Gln Gln Ala Pro Ile Pro Gln Gln Gln Pro
2020 2025 2030
Met Pro Gly Met Pro Arg Pro Val Met Ser Met Gln Ala Gln Ala Ala
2035 2040 2045
Val Ala Gly Pro Arg Met Pro Asn Val Gln Pro Asn Arg Ser Ile Ser
2050 2055 2060

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Pro Ser Ala Leu Gln Asp Leu Leu Arg Thr Leu Lys Ser Pro Ser Ser
065                 2070                2075                2080
Pro Gln Gln Gln Gln Gln Val Leu Asn Ile Leu Lys Ser Asn Pro Gln
                2085                2090                2095
Leu Met Ala Ala Phe Ile Lys Gln Arg Thr Ala Lys Tyr Val Ala Asn
            2100                2105                2110
Gln Pro Gly Met Gln Pro Gln Pro Gly Leu Gln Ser Gln Pro Gly Met
        2115                2120                2125
Gln Pro Gln Pro Gly Met His Gln Gln Pro Ser Leu Gln Asn Leu Asn
    2130                2135                2140
Ala Met Gln Ala Gly Val Pro Arg Pro Gly Val Pro Pro Pro Gln Pro
145                 2150                2155                2160
Ala Met Gly Gly Leu Asn Pro Gln Gly Gln Ala Leu Asn Ile Met Asn
                2165                2170                2175
Pro Gly His Asn Pro Asn Met Thr Asn Met Asn Pro Gln Tyr Arg Glu
            2180                2185                2190
Met Val Arg Arg Gln Leu Leu Gln His Gln Gln Gln Gln Gln Gln Gln
        2195                2200                2205
Gln Gln Gln Gln Gln Gln Gln Gln Asn Ser Ala Ser Leu Ala Gly Gly
    2210                2215                2220
Met Ala Gly His Ser Gln Phe Gln Gln Pro Gln Gly Pro Gly Gly Tyr
225                 2230                2235                2240
Ala Pro Ala Met Gln Gln Gln Arg Met Gln Gln His Leu Pro Ile Gln
                2245                2250                2255
Gly Ser Ser Met Gly Gln Met Ala Ala Pro Met Gly Gln Leu Gly Gln
            2260                2265                2270
Met Gly Gln Pro Gly Leu Gly Ala Asp Ser Thr Pro Asn Ile Gln Gln
        2275                2280                2285
Ala Leu Gln Gln Arg Ile Leu Gln Gln Gln Gln Met Lys Gln Gln Ile
    2290                2295                2300
Gly Ser Pro Gly Gln Pro Asn Pro Met Ser Pro Gln Gln His Met Leu
305                 2310                2315                2320
Ser Gly Gln Pro Gln Ala Ser His Leu Pro Gly Gln Gln Ile Ala Thr
                2325                2330                2335
Ser Leu Ser Asn Gln Val Arg Ser Pro Ala Pro Val Gln Ser Pro Arg
            2340                2345                2350
Pro Gln Ser Gln Pro Pro His Ser Ser Pro Ser Pro Arg Ile Gln Pro
        2355                2360                2365
Gln Pro Ser Pro His His Val Ser Pro Gln Thr Gly Thr Pro His Pro
    2370                2375                2380
Gly Leu Ala Val Thr Met Ala Ser Ser Met Asp Gln Gly His Leu Gly
385                 2390                2395                2400
Asn Pro Glu Gln Ser Ala Met Leu Pro Gln Leu Asn Thr Pro Asn Arg
                2405                2410                2415
Ser Ala Leu Ser Ser Glu Leu Ser Leu Val Gly Asp Thr Thr Gly Asp
            2420                2425                2430
Thr Leu Glu Lys Phe Val Glu Gly Leu
        2435                2440
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 813 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Ala Glu Ala Gly Gly Ala Gly Ser Pro Ala Leu Pro Pro Ala Pro
 1               5                  10                  15
```

```
Pro His Gly Ser Pro Arg Thr Leu Ala Thr Ala Ala Gly Ser Ser Ala
            20                  25                  30
Ser Cys Gly Pro Ala Thr Pro Val Ala Ala Ala Gly Thr Ala Glu Gly
        35                  40                  45
Pro Gly Gly Gly Gly Ser Ala Arg Ile Ala Val Lys Lys Ala Gln Leu
    50                  55                  60
Arg Ser Ala Pro Arg Ala Lys Lys Leu Glu Lys Leu Gly Val Tyr Ser
65                  70                  75                  80
Ala Cys Lys Ala Glu Glu Ser Cys Lys Cys Asn Gly Trp Lys Asn Pro
                85                  90                  95
Asn Pro Ser Pro Thr Pro Pro Arg Gly Asp Leu Gln Gln Ile Ile Val
            100                 105                 110
Ser Leu Thr Glu Ser Cys Arg Ser Cys Ser His Ala Leu Ala Ala His
        115                 120                 125
Val Ser His Leu Glu Asn Val Ser Glu Glu Glu Met Asp Arg Leu Leu
    130                 135                 140
Gly Ile Val Leu Asp Val Glu Tyr Leu Phe Thr Cys Val His Lys Glu
145                 150                 155                 160
Glu Asp Ala Asp Thr Lys Gln Val Tyr Phe Tyr Leu Phe Lys Leu Leu
                165                 170                 175
Arg Lys Ser Ile Leu Gln Arg Gly Lys Pro Val Val Glu Gly Ser Leu
            180                 185                 190
Glu Lys Lys Pro Pro Phe Glu Lys Pro Ser Ile Glu Gln Gly Val Asn
        195                 200                 205
Asn Phe Val Gln Tyr Lys Phe Ser His Leu Pro Ser Lys Glu Arg Gln
    210                 215                 220
Thr Thr Ile Glu Leu Ala Lys Met Phe Leu Asn Arg Ile Asn Tyr Trp
225                 230                 235                 240
His Leu Glu Ala Pro Ser Gln Arg Arg Leu Arg Ser Pro Asn Asp Asp
                245                 250                 255
Ile Ser Gly Tyr Lys Glu Asn Tyr Thr Arg Trp Leu Cys Tyr Cys Asn
            260                 265                 270
Val Pro Gln Phe Cys Asp Ser Leu Pro Arg Tyr Glu Thr Thr Lys Val
        275                 280                 285
Phe Gly Arg Thr Leu Leu Arg Ser Val Phe Thr Ile Met Arg Arg Gln
    290                 295                 300
Leu Leu Glu Gln Ala Arg Gln Lys Lys Asp Lys Leu Pro Leu Glu Lys
305                 310                 315                 320
Arg Thr Leu Ile Leu Thr His Phe Pro Lys Phe Leu Ser Met Leu Glu
                325                 330                 335
Glu Glu Val Tyr Ser Gln Asn Ser Pro Ile Trp Asp Gln Asp Phe Leu
            340                 345                 350
Ser Ala Ser Ser Arg Thr Ser Pro Leu Gly Ile Gln Thr Val Ile Ser
        355                 360                 365
Pro Pro Val Thr Gly Thr Ala Leu Phe Ser Ser Asn Ser Thr Ser His
    370                 375                 380
Glu Gln Ile Asn Gly Gly Arg Thr Ser Pro Gly Cys Arg Gly Ser Ser
385                 390                 395                 400
Gly Leu Glu Ala Asn Pro Gly Glu Lys Arg Lys Met Asn Asn Ser His
                405                 410                 415
Ala Pro Glu Glu Ala Lys Arg Ser Arg Val Met Gly Asp Ile Pro Val
            420                 425                 430
Glu Leu Ile Asn Glu Val Met Ser Thr Ile Thr Asp Pro Ala Gly Met
        435                 440                 445
Leu Gly Pro Glu Thr Asn Phe Leu Ser Ala His Ser Ala Arg Asp Glu
    450                 455                 460
Ala Ala Arg Leu Glu Glu Arg Arg Gly Val Ile Glu Phe His Val Val
465                 470                 475                 480
Gly Asn Ser Leu Asn Gln Lys Pro Asn Lys Lys Ile Leu Met Trp Leu
                485                 490                 495
Val Gly Leu Gln Asn Val Phe Ser His Gln Leu Pro Arg Met Pro Lys
            500                 505                 510
```

```
Glu Tyr Ile Thr Arg Leu Val Phe Asp Pro Lys His Lys Thr Leu Ala
        515                 520                 525
Leu Ile Lys Asp Gly Arg Val Ile Gly Gly Ile Cys Phe Arg Met Phe
    530                 535                 540
Pro Ser Gln Gly Phe Thr Glu Ile Val Phe Cys Ala Val Thr Ser Asn
545                 550                 555                 560
Glu Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn His Leu Lys Glu
                565                 570                 575
Tyr His Ile Lys His Glu Ile Leu Asn Phe Leu Thr Tyr Ala Asp Glu
            580                 585                 590
Tyr Ala Ile Gly Tyr Phe Lys Lys Gln Gly Phe Ser Lys Glu Ile Lys
        595                 600                 605
Ile Pro Lys Thr Lys Tyr Val Gly Tyr Ile Lys Asp Tyr Glu Gly Ala
    610                 615                 620
Thr Leu Met Gly Cys Glu Leu Asn Pro Gln Ile Pro Tyr Thr Glu Phe
625                 630                 635                 640
Ser Val Ile Ile Lys Lys Gln Lys Glu Ile Ile Lys Lys Leu Ile Glu
                645                 650                 655
Arg Lys Gln Ala Gln Ile Arg Lys Val Tyr Pro Gly Leu Ser Cys Phe
            660                 665                 670
Lys Asp Gly Val Arg Gln Ile Pro Ile Glu Ser Ile Pro Gly Ile Arg
        675                 680                 685
Glu Thr Gly Trp Lys Pro Ser Gly Lys Glu Lys Ser Lys Glu Pro Lys
    690                 695                 700
Asp Pro Glu His Val Tyr Ser Thr Leu Lys Asn Ile Leu Gln Gln Val
705                 710                 715                 720
Lys Asn His Pro Asn Ala Trp Pro Phe Met Glu Pro Val Lys Arg Thr
                725                 730                 735
Glu Ala Pro Gly Tyr Tyr Glu Val Ile Arg Phe Pro Met Asp Leu Lys
            740                 745                 750
Thr Met Ser Glu Arg Leu Arg Asn Arg Tyr Tyr Val Ser Lys Lys Leu
        755                 760                 765
Phe Met Ala Asp Leu Gln Arg Val Phe Thr Asn Cys Lys Glu Tyr Asn
    770                 775                 780
Pro Pro Glu Ser Glu Tyr Tyr Lys Cys Ala Ser Ile Leu Glu Lys Phe
785                 790                 795                 800
Phe Phe Ser Lys Ile Lys Glu Ala Gly Leu Ile Asp Lys
                805                 810
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
His Thr Lys Gly Cys Lys Arg Lys Thr Asn Gly Gly Cys Pro Val Cys
 1               5                  10                  15
Lys Gln Leu Ile Ala Leu Cys Cys Tyr His Ala Lys His Cys Gln Glu
            20                  25                  30
Asn Lys Cys Pro Val Pro Phe Cys Leu Asn Ile Lys His Asn Val Arg
        35                  40                  45
Gln Gln
    50
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2204 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACCCACTCCC CCCAGAGCCG ACCTGCAGCA AATAATTGTC AGTCTAACAG AATCCTGTCG 60
GAGTTGTAGC CATGCCCTAG CTGCTCATGT TTCCCACCTG GAGAATGTGT CAGAGGAAGA 120
AATGAACAGA CTCCTGGGAA TAGTATTGGA TGTGGAATAT CTCTTTACCT GTGTCCACAA 180
GGAAGAAGAT GCAGATACCA AACAAGTTTA TTTCTATCTA TTTAAGCTCT TGAGAAAGTC 240
TATTTTACAA AGAGGAAAAC CTGTGGTTGG AAGGCTCTTT GGAAAAGAAA CCCCCATTTG 300
AAAAACCTAG CATTGAACAG GGTGTGAATA ACTTTGTGCA GTACAAATTT AGTCACCTGC 360
CAGCAAAAAG AAAGGCAAAC CAATAGTTGA GTTGGCAAAA ATGTTCCTAA ACCGCATCAC 420
CTATTGGCAT CTGGAGGCAC CATCTCAACG AGACTGCGAT CTCCAATGAT GATATTCTGG 480
ATACAAAGAG AACTACACAA GGTGGCTGTG TTACTGCAAC GTGCCACAGT TCTGCGACAG 540
TCTACCTCGG TACGAAACCA CACAGGTGTT TGGGAGAACA TCGTTCGCTC GGTCTTCACT 600
GTTATGAGGC GACAACTCCT GGAACAAGCA AGACAGGAAA AAGATAAACT GCCTCTTGAA 660
AAACGAACTC TAATCCTCAC TCATTTCCCA AAATTTCTGT CCATGCTAGA AGAAGAAGTA 720
TATAGTCAAA ACTCTCCCAT CTGGGATCAC CATTTTCTCT CAGCCTCTTC CAGAACCAGC 780
CAGCTAGGCA TCCAAACAGT TATCAATCAC CTCCTGTGGC TGGGACAATT TCATACAATT 840
CAACCTCATC TTCCCTTGAG CAGCCAAACG CAGGGAGCAG CAGTCCTGCC TGCAAAGCCT 900
CTTCTGGACT TGAGGCAAAC CCAGGAGAAA AGAGGAAAAT GACTGATTCT CATGTTCTGG 960
AGGAGGCCAA GAAACCCCGA GTTATGGGGG ATATTCCGAT GGAATTAATC AACGAGGTTA 1020
TGTCTACCAT CACGGACCCT GCAGCAATGC TTGGACCAGA GACCAATTTT CTGTCAGCAC 1080
ACTCGGCCAG GGATGAGGCG GCAAGGTTGG AAGAGCGCAG GGGTGTAATT GAATTTCACG 1140
TGGTTGGCAA TTCCCTCAAC CAGAAACCAA ACAAGAAGAT CCTGATGTGG CTGGTTGGCC 1200
TACAGAACGT TTTCTCCCAC CAGCTGCCCC GAATGCCAAA AGAATACATC ACACGGCTCG 1260
TCTTTGACCC GAAACACAAA ACCCTTGCTT TAATTAAAGA TGGCCGTGTT ATTGGTGGTA 1320
TCTGTTTCCG TATGTTCCCA TCTCAAGGAT TCACAGAGAT TGTCTTCTGT GCTGTAACCT 1380
CAAATGAGCA AGTCAAGGGC TATGGAACAC ACCTGATGAA TCATTTGAAA GAATATCACA 1440
TAAAGCATGA CATCCTGAAC TTCCTCACAT ATGCAGATGA ATATGCAATT GGATACTTTA 1500
AGAAACAGGG TTTCTCCAAA GAAATTAAAA TACCTAAAAC CAAATATGTT GGCTATATCA 1560
AGGATTATGA AGGAGCCACT TTAATGGGAT GTGAGCTAAA TCCACGGATC CCGTACACAG 1620
AATTTTCTGT CATCATTAAA AAGCAGAAGG AGATAATTAA AAAACTGATT GAAAGAAAAC 1680
AGGCACAAAT TCGAAAAGTT TACCCTGGAC TTTCATGTTT TAAAGATGGA GTTCGACAGA 1740
TTCCTATAGA AAGCATTCCT GGAATTAGAG AGACAGGCTG GAAACCGAGT GGAAAAGAGA 1800
AAAGTAAAGA GCCCAGAGAC CCTGACCAGC TTTACAGCAC GCTCAAGAGC ATCCTCCAGC 1860
AGGTGAAGAG CCATCAAAGC GCTTGGCCCT TCATGGAACC TGTGAAGAGA ACAGAAGCTC 1920
CAGGATATTA TGAAGTTATA AGGTCCCCCA TGGATCTCAA AACCATGAGT GAACGCCTCA 1980
AGAATAGGTA CTACGTGTCT AAGAAATTAT TCATGGCAGA CTTACAGCGA GTCTTTACCA 2040
ATTGCAAAGA GTACAACGCC CCTGAGAGTG AATACTACAA ATGTGCCAAT ATCCTGGAGA 2100
AATTCTTCTT CAGTAAAATT AAGGAAGCTG GATTAATTGA CAAGTGATTT TTTTTCCCCC 2160
TCTGCTTCTT AGAAACTCAC CAAGCAGTGT GCCTAAAGCA AGGT 2204

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2093 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTCCGGC GAAACCACTC ATGTCTTTGG GCGAAGCCTT CTCCGGTCCA TTTTCACCGT 60
TACCCGCCGG CAGCTGCTGG AAAAGTTCCG AGTGGAGAAG GACAAATTGG TGCCCGAGAA 120
GAGGACCCTC ATCCTCACTC ACTTCCCCAA GTAAGGCTCC TTCTGGCCTA CCAGGATTTG 180
GCCCCAAGTT CACATCCTCC CTGTTGTCCC CTTTTTTCCA GGAAGGCTTC CTGGATTGGT 240
CCCTCCTCTC CCTCCATGGG CCTTTTGGGA TCTGGGCGTC TACCTGGCAG ACTTGCCCAT 300
GGCCCAGAAG CAACTTGCTA GTACTAGTCT GGGGATGGCA GATTCCTGTC CATGCTGGAG 360
GAGGAGATCT ATGGGGCAAA CTCTCCAATC TGGGAGTCAG GCTTCACCAT GCCACCCTCA 420

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GAGGGGACAC AGCTGGTTCC CCGGCCAGCT TCAGTCAGTG CAGCGGTTGT TCCCAGCACC    480
CCCATCTTCA GCCCCAGCAT GGGTGGGGGC AGCAACAGCT CCCTGAGTCT GGATTCTGCA    540
GGGGCCGAGC CTATGCCAGG CGAGAAGAGG ACGCTCCCAG AGAACCTGAC CCTGGAGGAT    600
GCCAAGCGGC TCCGTGTGAT GGGTGACATC CCCATGGAGC TGGTCAATGA GGTCATGCTG    660
ACCATCACTG ACCCTGCTGC CATGCTGGGG CCTGAGACGA GCCTGCTTTC GGCCAATGCG    720
GCCCGGGATG AGACAGCCCG CCTGGAGGAG CGCCGCGGCA TCATCGAGTT CCATGTCATC    780
GGCAACTCAC TGACGCCCAA GGCCAACCGG CGGGTGTTGC TGTGGCTCGT GGGGCTGCAG    840
AATGTCTTTT CCCACCAGCT GCCGCGCATG CCTAAGGAGT ATATCGCCCG CCTCGTCTTT    900
GACCCGAAGC ACAAGACTCT GGCCTTGATC AAGGATGGGC GGGTCATCGG TGGCATCTGC    960
TTCCGCATGT TTCCCACCCA GGGCTTCACG GAGATTGTCT TCTGTGCTGT CACCTCGAAT   1020
GAGCAGGTCA AGGGTTATGG GACCCACCTG ATGAACCACC TGAAGGAGTA TCACATCAAG   1080
CACAACATTC TCTACTTCCT CACCTACGCC GACGAGTACG CCATCGGCTA CTTCAAAAAG   1140
CAGGGTTTCT CCAAGGACAT CAAGGTGCCC AAGAGCCGCT ACCTGGGCTA CATCAAGGAC   1200
TACGAGGGAG CGACGCTGAT GGAGTGTGAG CTGAATCCCC GCATCCCCTA CACGGAGCTG   1260
TCCCACATCA TCAAGAAGCA GAAAGAGATC ATCAAGAAGC TGATTGAGCG CAAACAGGCC   1320
CAGATCCGCA AGGTCTACCC GGGGCTCAGC TGCTTCAAGG AGGGCGTGAG GCAGATCCCT   1380
GTGGAGAGCG TTCCTGGCAT TCGAGAGACA GGCTGGAAGC CATTGGGGAA GGAGAAGGGG   1440
AAGGAGCTGA AGGACCCCGA CCAGCTCTAC ACAACCCTCA AAAACCTGCT GGCCCAAATC   1500
AAGTCTCACC CCAGTGCCTG GCCCTTCATG GAGCCTGTGA AGAAGTCGGA GGCCCCTGAC   1560
TACTACGAGG TCATCCGCTT CCCCATTGAC CTGAAGACCA TGACTGAGCG GCTGCGAAGC   1620
CGCTACTACG TGACCCGGAA GCTCTTTGTG GCCGACCTGC AGCGGGTCAT CGCCAACTGT   1680
CGCGAGTACA ACCCCCCGGA CAGCGAGTAC TGCCGCTGTG CCAGCGCCCT GGAGAAGTTC   1740
TTCTACTTCA AGCTCAAGGA GGGAGGCCTC ATTGACAAGT AGGCCCATCT TTGGGCCGCA   1800
GCCCTGACCT GGAATGTCTC CACCTCGGAT TCTGATCTGA TCCTTAGGGG GTGCCCTGGC   1860
CCCACGGACC CGACTCAGCT TGAGACACTC CAGCCAAGGG TCCTCCGGAC CCGATCCTGC   1920
AGCTCTTTCT GGACCTTCAG GCACCCCCAA GCGTGCAGCT CTGTCCCAGC CTTCACTGTG   1980
TGTGAGAGGT CTCCTGGGTT GGGGCCCAGC CCCTCTAGAG TAGCTGGTGG CCAGGGATGA   2040
ACCTTGCCCA GCCGTGGTGG CCCCCAGGCC TGGTCCCCAA GAGCCCGGAA TTC          2093
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9046 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
CCTTGTTTGT GTGCTAGGCT GGGGGGGAGA GAGGGCGAGA GAGAGCGGGC GAGAGTGGGC     60
AAGCAGGACG CCGGGCTGAG TGCTAACTGC GGGACGCAGA GAGTGCGGAG GGGAGTCGGG    120
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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7326 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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CAGGTGCGAT CTCCAGCCCC TGTGCAGTCT CCACGGCCCC AATCCCAACC TCCACATTCC 7080
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ACCCCTCACC CTGGACTCGC AGTCACCATG GCCAGCTCCA TGGATCAGGG ACACCTGGGG 7200
AACCCTGAAC AGAGTGCAAT GCTCCCCCAG CTGAATACCC CCAACAGGAG CGCACTGTCC 7260
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TTGTAG 7326

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2499 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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CATGGTTTTG AGATCCATGG GGGACCTTAT AACTTCATAA TATCCTGGAG CTTCTGTTCT 240
CTTCACAGGT TCCATGAAGG GCCAAGCGCT TTGATGGCTC TTCACCTGCT GGAGGATGCT 300
CTTGAGCGTG CTGTAAAGCT GGTCAGGGTC TCTGGGCTCT TTACTTTTCT CTTTTCCACT 360
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(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2442 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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GAAAACAATC TCTGTGAATC CCTGGGATGG AAACATCCGG AAACAGATAC CACCAATGAC 840
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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCCGCCAGCC TCGGCCATGC 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2442 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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TATTATGAAG TTATAAGGTT CCCCATGGAT CTGAAAACCA TGAGTGAACG CCTCAGGAAC   2280
AGGTACTATG TGTCTAAGAA GTTATTCATG GCGGACTTGC AACGAGTGTT CACCAACTGC   2340
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What is claimed is:

1. A purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones.

2. The protein of claim 1 consisting of the amino acid sequence of SEQ ID NO:1.

3. The protein of claim 1 comprising the amino acid sequence of SEQ ID NO:2.

4. The protein of claim 1, which also binds to the amino acid sequence of SEQ ID NO:3 on a p300 cellular protein and to amino acid residues 1805-1854 of a CBP cellular protein (SEQ ID NO:9).

5. A fragment of the protein of claim 1 having histone acetyltransferase activity.

6. A polypeptide consisting of the amino acid sequence of SEQ ID NO: 2.

7. A fragment of the protein of claim 1 which binds to the amino acid sequence of SEQ ID NO: 3 on the p300 cellular protein and the amino acid sequence of SEQ ID NO:9 on the CBP cellular protein.

8. A polypeptide consisting of the amino acid sequence of SEQ ID NO:4.

9. A nucleic acid consisting of the nucleotide sequence of SEQ ID NO:10.

10. A nucleic acid having a nucleotide sequence which encodes the protein of claim 1.

11. A nucleic acid having a nucleotide sequence which encodes the protein of claim 2.

12. A nucleic acid having a nucleotide sequence which encodes the protein of claim 3.

13. A nucleic acid consisting of the nucleotide sequence which encodes the protein of claim 4.

14. A nucleic acid complementary to and which selectively hybridizes with the nucleic acid of claim 11 under stringent hybridization conditions.

15. A fragment of the nucleic acid of claim 9, which encodes a polypeptide that acetylates histones.

16. A fragment of the nucleic acid of claim 9, which encodes a polypeptide which binds to the amino acid sequence of SEQ ID NO:3 on the p300 cellular protein and the amino acid sequence of SEQ ID NO:9 on the CBP cellular protein.

17. A purified antibody which specifically binds the protein of claim 1.

18. A purified antibody which specifically binds the protein of claim 2.

19. A purified antibody which specifically binds the protein of claim 3.

20. A purified antibody which specifically binds the protein of claim 4.

21. An assay for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of P/CAF comprising:

a) contacting the substance with a system in which histone acetylation by P/CAF can be determined;

b) determining the amount of histone acetylation by P/CAF in the presence of the substance; and

c) comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased or increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of P/CAF.

22. An assay for screening substances for the ability to inhibit binding of P/CAF to p300/CBP comprising:

a) contacting the substance with a system in which the P/CAF binding of P300/CBP can be determined;

b) determining the amount of P/CAF binding of p300/CBP in the presence of the substance; and

c) comparing the amount of binding of P/CAF to p300/CBP in the presence of the substance with the amount of binding of P/CAF to p300/CBP in the absence of the substance, a decreased amount of binding of P/CAF to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of P/CAF to p300/CBP.

23. The method of claim 22, wherein the system consists of a cell free reaction mixture comprising a fragment of the p300 protein comprising amino acid residues 1767-1816 (SEQ ID NO:3) and the protein of claim 4.

24. The method of claim 22, wherein the system consists of a cell free reaction mixture comprising a fragment of the CBP protein comprising amino acid residues 1805-1854 (SEQ ID NO:9) and the protein of claim 4.

25. The method of claim 22, wherein the system consists of a cell extract produced from cells producing both p300 and P/CAF.

26. An assay for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of p300/CBP comprising:

a) contacting the substance with a system in which histone acetylation by p300/CBP can be determined;

b) determining the amount of histone acetylation by p300/CBP in the presence of the substance; and

c) comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, a decreased or increased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of p300/CBP.

27. An assay for screening substances for the ability to inhibit binding of a DNA-binding transcription factor to p300/CBP comprising:

a) contacting the substance with a system in which the DNA-binding transcription factor binding of P300/CBP can be determined;

b) determining the amount of DNA-binding transcription factor binding of p300/CBP in the presence of the substance; and

c) comparing the amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance with the amount of binding of DNA-binding transcription factor to p300/CBP in the absence of the substance, a decreased amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of DNA-binding transcription factor to p300/CBP.

28. The method of claim 27, wherein the system consists of a cell free reaction mixture comprising a DNA-binding transcription factor and p300/CBP.

29. The method of claim 27, wherein the system consists of a cell free reaction mixture comprising a fragment of the CBP protein comprising a DNA-binding transcription factor and p300/CBP.

30. The method of claim 27, wherein the system consists of a cell extract produced from cells producing both a DNA-binding transcription factor and p300/CBP.

31. The method of claim 27, wherein the DNA-binding transcription factor is selected from the group consisting of a nuclear hormone receptor, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YY1, Sap-1a, c-Fos, MyoD and SRC-1.

32. A method for inhibiting the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

33. The method of claim 32, wherein the substance can inhibit the transcription modulating activity of P/CAF by preventing the binding of P/CAF to p300/CBP.

34. A method for stimulating the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

35. The method of claim 34, wherein the substance can stimulate the transcription modulating activity of P/CAF by promoting the binding of P/CAF to p300/CBP.

36. The method of claim 34, wherein the substance can stimulate the transcription modulating activity of P/CAF by stimulating the histone acetlytransferase activity of P/CAF.

37. A method for inhibiting the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

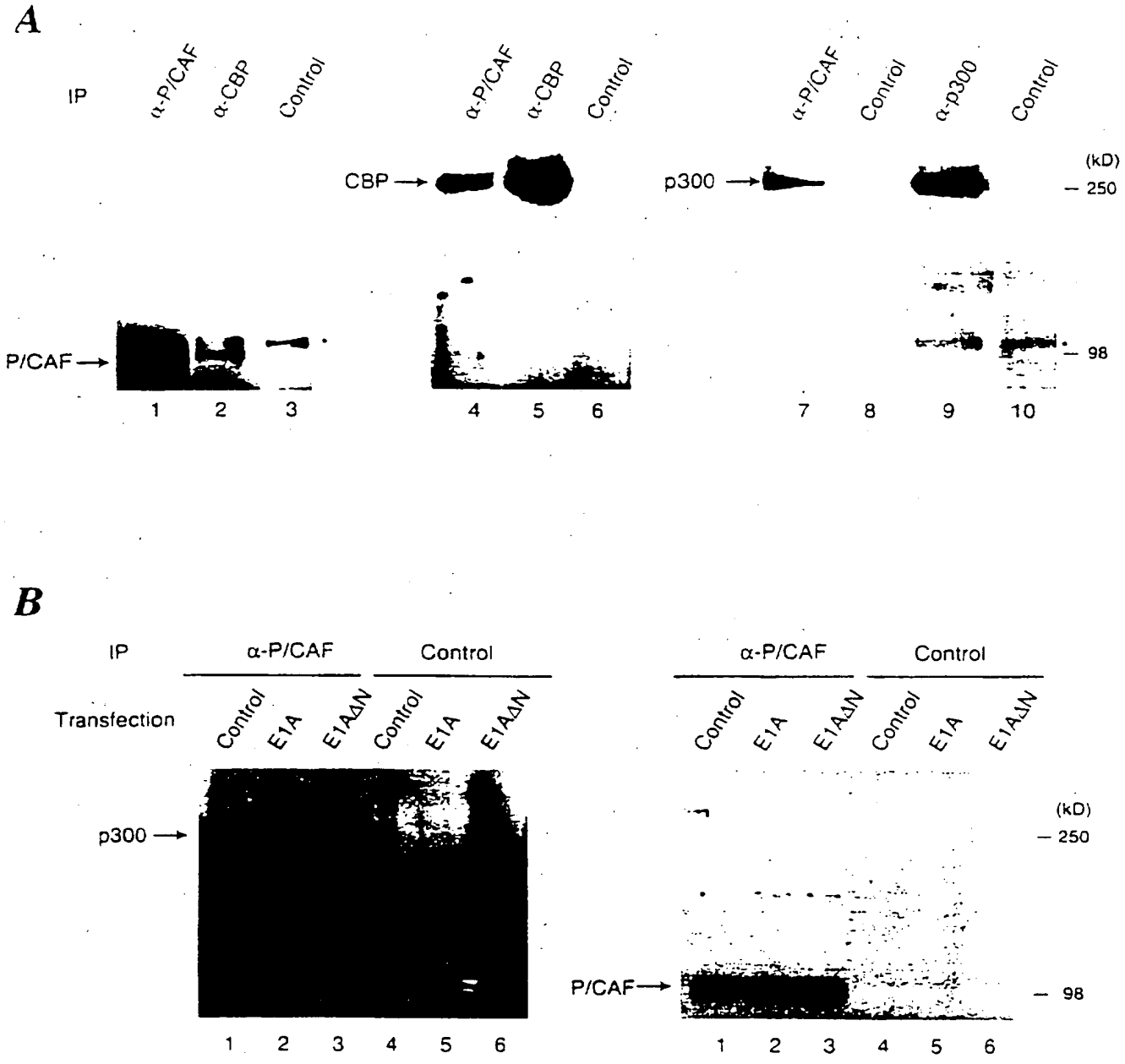
38. The method of claim 37, wherein the substance can inhibit the transcription modulating activity of p300/CBP by preventing the binding of a DNA-binding transcription factor to p300/CBP.

39. The method of claim 38, wherein the DNA-binding transcription factor is selected from the group consisting of a nuclear hormone receptor, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YY1, Sap-1a, c-Fos, MyoD and SRC-1.

40. The method of claim 37, wherein the substance is an antibody which binds p300/CBP.

41. A method for stimulating the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

42. The method of claim 41, wherein the substance can stimulate the histone acetyltransferase activity of p300/CBP by promoting the binding of a DNA-binding transcription factor to p300/CBP.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Control
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CBP
p300
(kD)
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P/CAF
98
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Control
α-P/CAF
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Transfection
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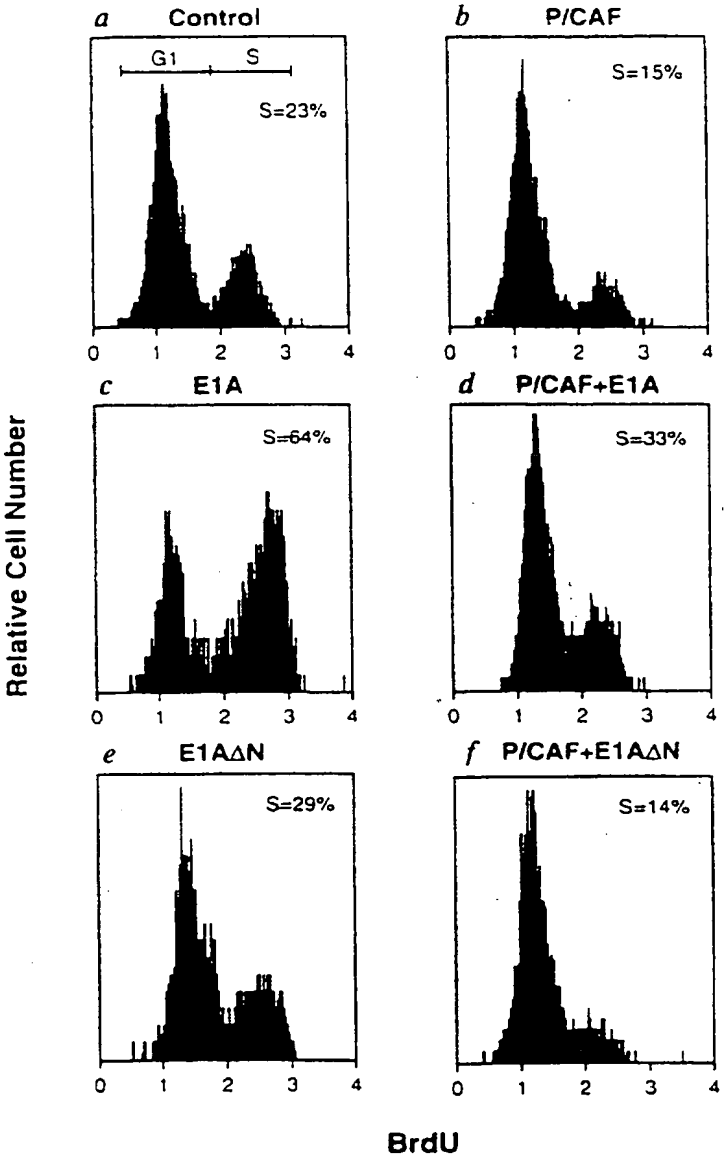
1 2 3 4 5 6

Fig. 1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S=23%
b P/CAF
S=15%
c E1A
S=64%
d P/CAF+E1A
S=33%
e E1AΔN
S=29%
f P/CAF+E1AΔN
S=14%
0 1 2 3 4
Relative Cell Number
BrdU

Fig. 2

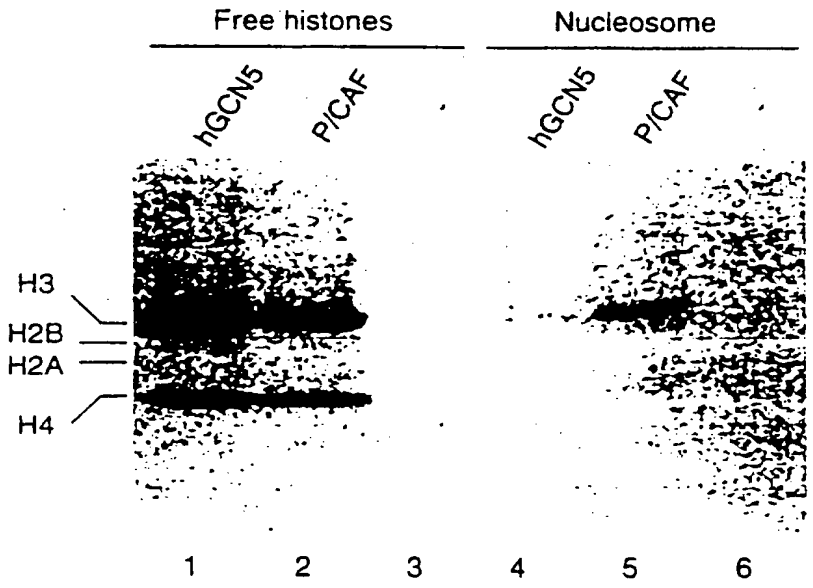
Free histones
Nucleosome
hGCN5
P/CAF
hGCN5
P/CAF
H3
H2B
H2A
H4
1
2
3
4
5
6

Fig. 3

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NAKATANI, Yoshihiro [JP/US]; 4624 Edgefield Road, Bethesda, MD 20814 (US). HOWARD, Bruce, H. [US/US]; 8715 Fallen Oak Drive, Bethesda, MD 20817 (US).

(74) Agents: MILLER, Mary, L. et al.; Needle & Rosenberg, Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303 (US).

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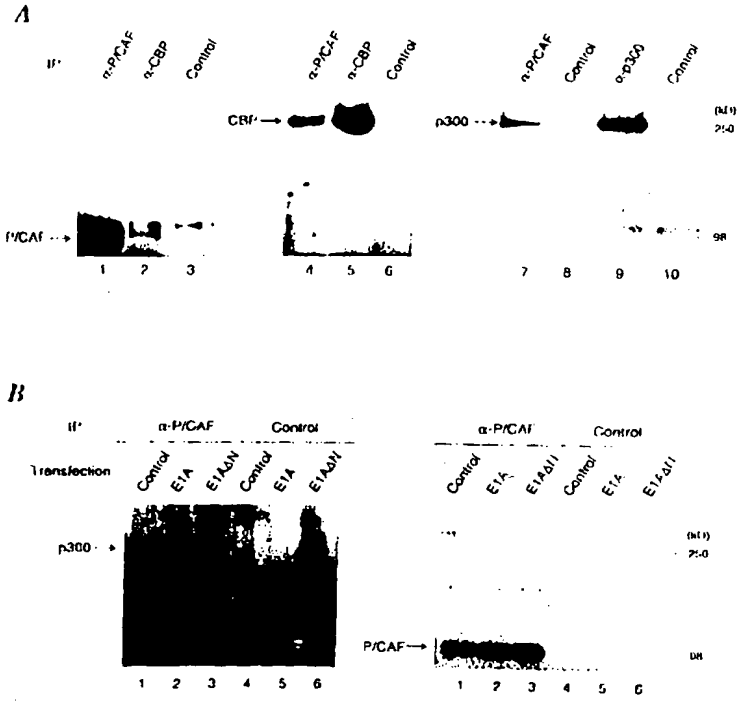
(57) Abstract

The present invention provides a purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones and which also binds to the p300/CBP cellular protein. The present invention further provides a nucleic acid encoding the P/CAF protein as well as a vector containing the nucleic acid and a host for the vector. A purified antibody which specifically binds the P/CAF protein is also provided. Also provided are methods of screening for compounds that inhibit or stimulate the transcription modulating and histone acetyltransferase activity of P/CAF and p300/CBP.



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INTERNATIONAL SEARCH REPORT

.nal Application No
PCT/US 97/12877

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMBL EST, Accession numberN39522 Sequence no yv27b08.s1 Homo sapiens cDNA clone 243927 3' 25 January 1996 XP002050402 see the whole document ---	1
A	GEORGAKOPOULOS, T. & THIREOS, G.: EMBO JOURNAL., vol. 11, 1992, EYNSHAM, OXFORD GB, pages 4145-4152, XP002050399 see the whole document --- -/--	1

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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1

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1997	14.01.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Chambonnet, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	YANG, X.Y. ET AL.: "A p300-CBP-associated factor that competes with the adenoviral oncoprotein E1A" NATURE., vol. 382, no. 8589, 25 July 1996, LONDON GB, pages 319-324, XP002050400 see the whole document	1
P,X	OGRYZKO, V.V. ET AL.: "The transcriptional coactivators p300 and CBP are histone acetyltransferases" CELL, vol. 87, no. 5, November 1996, NA US, pages 953-959, XP002050401 see the whole document	1
P,X	EMBL EST, Accession number U57316, Sequence reference human GCN5 (hGCN5) complete cds. 26 august 1996 XP002050403 see the whole document	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/12877

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 32 to 42 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the composition.

CORRECTED VERSION*

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
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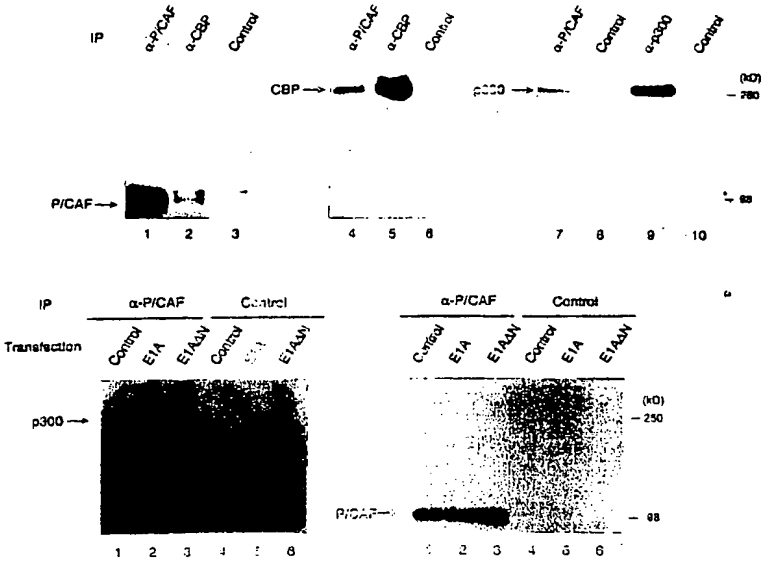

*(Referred to in PCT Gazette No. 17/1998, Section II)

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P300/CBP-ASSOCIATED TRANSCRIPTIONAL CO-FACTOR P/CAF AND USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention provides a transcriptional co-factor, p300/CBP-associated factor (P/CAF), which modulates transcription through binding to the cellular transcription co-factors p300 and CBP and through acetylation of histones. Also provided are methods for screening for the presence of P/CAF and for substances which alter the transcription modulating effect and growth regulatory activity of P/CAF.

Background Art

Cellular proteins p300 and CBP are global transcriptional coactivators that are involved in the regulation of various DNA-binding transcriptional factors (Janknecht and Hunter, 1996). Recently, p300 was found to be very closely related to CBP, a factor that binds selectively to the protein kinase A-phosphorylated form of CREB (3-5). Cellular factors p300 and CBP exhibit strong amino acid sequence similarity and share the capacity to bind both CREB and E1A (6-8). Although neither p300 nor CBP by itself binds to DNA, each can be recruited to promoter elements via interaction with sequence-specific activators and functions to be a transcriptional adaptor. For simplicity, p300 and CBP will be termed p300/CBP in the context of discussing their shared functional properties.

p300/CBP is a large protein consisting of over 2,400 amino acids, known to interact with a variety of DNA-binding transcriptional factors including nuclear hormone receptors (13,57), CREB (3,4, 7), c-Jun/v-Jun (9,11), YY1 (10), c-Myb/v-Myb (12,58), Sap-1a (59), c-Fos (11) and MyoD (60). DNA-binding factors recruit p300/CBP not only by direct but also indirect interactions through cofactors; for example, nuclear hormone receptors recruit p300/CBP directly as well as through indirect interactions, via SRC-1, which stimulates transcription by binding to various nuclear hormone receptors (13,61).

The transforming proteins encoded by adenovirus and several other small DNA tumor viruses disturb host cell growth control by interacting with cellular factors that normally function to repress cell proliferation. One of the most intensively studied of these viral proteins, the product of the adenovirus E1A gene, is itself sufficient for transformation (1). E1A transforming activity resides in two distinct domains, the targets of which include p300/CBP and products of the retinoblastoma (RB) susceptibility gene family (1,2). Interactions of E1A with p300/CBP and RB are thought to influence functionally distinct growth regulatory pathways, allowing the two domains to contribute additively to transformation (1).

The paradigm for how E1A and functionally related viral proteins perturb cell growth regulation derives in large part from studies on their interactions with RB (1,2). The molecular function of E1A is based on its capacity to interfere with cellular protein-protein interactions. Since both E1A and various cellular targets bind to a site in RB termed the pocket domain (2), E1A can competitively disrupt the complex formation between RB and its cellular targets.

The second cellular factor implicated in E1A-dependent transformation, p300, is believed to inhibit G0/G1 exit, to activate certain enhancers, and to stimulate differentiation (1,2). E1A inhibits the p300/CBP-mediated transcriptional activation of many promoters (14). In one case that has been examined, the complex of p300 and YY1, E1A inhibits transcription without disrupting the complex (10).

The present invention provides a cellular protein designated P/CAF which binds to p300/CBP and plays an important role in both transcription and cell cycle regulation associated with a histone acetyltransferase activity. The present invention also provides a histone acetyltransferase activity in the p300/CBP cellular protein, thus providing targets for modulating transcription and cell cycle regulation in cells.

SUMMARY OF THE INVENTION

The present invention provides a purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones and which also binds to the p300/CBP cellular protein.

The present invention further provides a nucleic acid encoding the P/CAF protein as well as a vector containing the nucleic acid and a host for the vector. A purified antibody which specifically binds the P/CAF protein is also provided.

In addition, also provided is a bioassay for screening substances for the ability to inhibit the transcription modulating activity of P/CAF and/or histone acetyltransferase activity, comprising contacting the substance with a system in which histone acetylation by P/CAF can be determined; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit the transcription modulating activity and/or histone acetyltransferase activity of P/CAF.

Furthermore, the present invention provides a bioassay for screening substances for the ability to inhibit the transcription modulating activity and/or histone acetyltransferase activity of P/CAF comprising contacting the substance with a system in which the p300 binding of P/CAF can be determined; determining the amount of p300 binding of P/CAF in the presence of the substance; and comparing the amount of p300 binding of P/CAF in the presence of the substance with the amount of p300 binding of P/CAF in the absence of the substance, a decreased amount of p300 binding of P/CAF in the presence of the substance indicating a substance that can inhibit the transcription modulating activity and/or histone acetyltransferase activity of P/CAF.

Also provided is a method for determining the amount of P/CAF in a biological sample comprising contacting the biological sample with a polypeptide comprising the amino acid sequence of SEQ ID NO:3 under conditions whereby a P/CAF/p300 complex can be formed; and determining the amount of the P/CAF/p300 complex, the amount of the complex indicating the amount of P/CAF in the sample.

The present invention additionally provides a method for determining the amount of P/CAF in a biological sample comprising contacting the biological sample with an antibody which specifically binds P/CAF under conditions whereby a P/CAF/antibody complex can be formed; and determining the amount of the P/CAF/antibody complex, the amount of the complex indicating the amount of P/CAF in the sample.

Also provided herein is an assay for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of P/CAF, comprising: contacting the substance with a system in which histone acetylation by P/CAF can be determined; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased or increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of P/CAF.

The present invention further provides an assay for screening substances for the ability to inhibit binding of P/CAF to p300/CBP comprising: contacting the substance with a system in which the P/CAF binding of P300/CBP can be determined; determining the amount of P/CAF binding of p300/CBP in the presence of the substance; and comparing the amount of binding of P/CAF to p300/CBP in the presence of the substance with the amount of binding of P/CAF to p300/CBP in the absence of the substance, a decreased amount of binding of P/CAF to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of P/CAF to p300/CBP.

In addition, an assay is provided for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of p300/CBP, comprising: contacting the substance with a system in which histone acetylation by p300/CBP can be determined; determining the amount of histone acetylation by p300/CBP in the presence of the substance; and comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, a decreased or increased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of p300/CBP.

Furthermore, the present invention provides an assay for screening substances for the ability to inhibit binding of a DNA-binding transcription factor to p300/CBP comprising: contacting the substance with a system in which the DNA-binding transcription factor binding of P300/CBP can be determined; determining the amount of DNA-binding transcription factor binding of p300/CBP in the presence of the substance; and comparing the amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance with the amount of binding of DNA-binding transcription factor to p300/CBP in the absence of the substance, a decreased amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of DNA-binding transcription factor to p300/CBP.

A method is also provided for inhibiting the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

Also provided in the present invention is a method for stimulating the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

Furthermore, the present invention provides a method for inhibiting the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

Finally, the present invention additionally provides a method for stimulating the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1A-B. Fig 1A: P/CAF-p300/CBP interaction *in vivo*. Cell extract was immunoprecipitated with rabbit anti-P/CAF (lanes 1, 4, and 7), rabbit anti-CBP (lanes 2 and 5), and mouse anti-p300 (lane 9) antibodies. For controls, cell extract was precipitated with rabbit control IgG (lanes 3, 6, and 8) or mouse anti-HA monoclonal antibody (lane 10). The precipitates were analyzed by immunoblotting with anti-P/CAF (lanes 1-3), anti-CBP (lanes 4-6), and anti-p300 (lanes 7-10) antibodies. The positions of non-specific bands are indicated by asterisks. Fig. 1B: E1A inhibits the P/CAF-p300 interaction *in vivo*. Osteosarcoma cells were transfected with either control vector (lanes 1 and 4) or E1A- (lanes 2 and 5) or E1AΔN- (lanes 3 and 6) expression vectors. Extract from the transfected subpopulation was immunoprecipitated with anti-P/CAF (lanes 1-3) or control (lanes 4-6) IgG. The precipitates were analyzed by immunoblotting with anti-p300 and anti-P/CAF.

Figs. 2A-F. P/CAF and E1A mediate antagonistic effects on cell cycle progression. HeLa cells (ATCC accession number CCL 2) were transfected by electroporation with 7 μg of P/CAF-expression plasmid and/or 3 μg of the full-length or the N-terminally deleted (Δ2-36) E1A 12S-expression plasmid as indicated in the figure. These plasmids were constructed by subcloning FLAG-P/CAF and E1A cDNAs into

pCX (34) and pcDNAI (Invitrogen), respectively. All samples, in addition, contained 1 μg of sorting plasmid (pCMV-IL2R) (31) and carrier plasmid (pCX) to normalize the total amount of DNA to 11 μg. After transfection, cells were incubated in Dulbecco's modified Eagle's medium with 10% fetal bovine calf serum for 12 hours and subsequently labeled in medium containing 10 μM bromo-deoxyuridine (BrdU) for 30 min. Subsequently, the transfected subpopulation was purified by magnetic affinity cell sorting and nuclei were analyzed by dual parameter flow cytometry as described (32). Histograms show percentages of cells in G1 and S phases. Abscissa values represent fluorescence intensity of bound anti-BrdU antibodies in log scale.

Fig. 3. Histone acetyltransferase activity of P/CAF. Activity of hGCN5 (lanes 1 and 4) and P/CAF (lanes 2 and 5) that acetylates free histones (lanes 1-3) or histones in the nucleosome core particle (35) (lanes 4-6) was measured as described (36). Each reaction contains 0.3 pmol of affinity purified FLAG-hGCN5 or FLAG-P/CAF, 4 pmol of the histone octamer or the nucleosome core particle and 10 pmol of [1-^{14}C]acetyl-CoA. Note that the histone octamer dissociates into dimers or tetramers under assay conditions. Acetylated histones were detected by autoradiography after separation by SDS-PAGE. The bands corresponding to acetylated histones H3 and H4 are indicated by arrows.

DETAILED DESCRIPTION OF THE INVENTION

As used in the specification and in the claims, "a" can mean one or more, depending upon the context in which it is used.

P/CAF protein and fragments

The present invention provides a purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones. The P/CAF protein can also bind to the amino acid region of SEQ ID NO:3

(amino acid (aa) residues 1753 - 1966) of the cellular transcriptional factor, p300 (which has the complete amino acid sequence of SEQ ID NO:6 and the nucleotide sequence of SEQ ID NO:12), and the amino acid region of SEQ ID NO:6 (amino acid residues 1805 - 1854) of the cellular transcriptional factor, CBP (which has the complete amino acid sequence of SEQ ID NO:7 and the nucleotide sequence of SEQ ID NO:13). The P/CAF protein can be defined by any one or more of the typically used parameters. Examples of these parameters include, but are not limited to molecular weight (calculated or empirically determined), isoelectric focusing point, specific epitope(s), complete amino acid sequence, sequence of a specific region (e.g., N-terminus) of the amino acid sequence and the like.

For example, The P/CAF protein can consist of the amino acid sequence of SEQ ID NO:1 or the P/CAF protein can comprise the amino acid sequence of SEQ ID NO:2 which represents the carboxy terminal end of the P/CAF protein and contains the histone acetyltransferase activity, or the amino acid sequence of SEQ ID NO:4, which represents the amino terminal end of the P/CAF protein, containing the binding site for p300/CBP. Because the amino-terminal region is specific for P/CAF it can be used to define and identify P/CAF.

As used herein, "purified" refers to a protein (polypeptide, peptide, etc.) that is sufficiently free of contaminants or cell components with which it normally occurs to distinguish it from the contaminants or other components of its natural environment. The purified protein need not be homogeneous, but must be sufficiently free of contaminants to be useful in a clinical or research setting, for example, in an assay for detecting antibodies to the protein. Greater levels of purity can be obtained using methods derived from well known protocols. Specific methods for purifying P/CAF proteins are known in the art.

As will be appreciated by those skilled in the art, the invention also includes those P/CAF polypeptides having slight variations in amino acid sequence which yield polypeptides equivalent to the P/CAF protein defined herein. Such variations may arise

naturally as allelic variations (*e.g.*, due to genetic polymorphism) or may be produced by human intervention (*e.g.*, by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion and substitution mutants. Minor changes in amino acid sequence are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules. Substitutions may be designed based on, for example, the model of Dayhoff, *et al.* (37). These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations.

Modifications to any of the P/CAF proteins or fragments can be made, while preserving the specificity and activity (function) of the native protein or fragment thereof. As used herein, "native" describes a protein that occurs in nature. The modifications contemplated herein can be conservative amino acid substitutions, for example, the substitution of a basic amino acid for a different basic amino acid. Modifications can also include creation of fusion proteins with epitope tags or known recombinant proteins or genes encoding them created by subcloning into commercial or non-commercial vectors (e.g., polyhistidine tags, flag tags, myc tag, glutathione-S-transferase [GST] fusion protein, *xylE* fusion reporter construct). Furthermore, the modifications can be such as do not affect the function of the protein or the way the protein accomplishes that function (e.g., its secondary structure or the ultimate result of the protein's activity). These products are equivalent to the P/CAF protein. The means for determining the function, way and result parameters are well known.

Having provided an example of a purified P/CAF protein, the invention also enables the purification of P/CAF homologs from other species and allelic variants from individuals within a species. For example, an antibody raised against the exemplary human P/CAF protein can be used routinely to screen preparations from different humans for allelic variants of the P/CAF protein that react with the P/CAF protein-specific antibody. Similarly, an antibody raised against an epitope, for example, from a conserved amino acid region of the human P/CAF protein can be used to routinely screen for homologs of the P/CAF protein in other species. A P/CAF protein can be

routinely identified in and obtained from other species and from individuals within a species using the methods taught herein and others known in the art. For example, given the present sequence, the DNA encoding a conserved amino acid sequence can be used to probe genomic DNA or DNA libraries of an organism to predictably obtain the P/CAF gene for that organism. The gene can then be cloned and expressed as the P/CAF protein and purified according to any of a number of routine, predictable methods. An example of the routine protein purification methods available in the art can be found in Pei et al. (38).

A purified polypeptide fragment of the P/CAF protein is also provided. The term "fragment" as used herein regarding a P/CAF protein, means a molecule of at least five contiguous amino acids of P/CAF protein that has at least one function shared by P/CAF protein or a region thereof. These functions can include antigenicity, binding capacity, acetyltransferase activity and structural roles, among others. The P/CAF fragment can be specific for a recited source. As used herein to describe an amino acid sequence (protein, polypeptide, peptide, etc.), "specific" means that the amino acid sequence is not found identically in any other source. The determination of specificity is made routine by the availability of computerized amino acid sequence databases and sequence comparison programs, wherein an amino acid sequence of almost any length can be quickly and reliably checked for the existence of identical sequences. If an identical sequence is not found, the protein is "specific" for the recited source. For example, a P/CAF fragment can be species-specific (e.g., found in the P/CAF protein of humans, but not of other species).

A fragment of the P/CAF protein having histone acetyltransferase activity can consist of the amino acid sequence of SEQ ID NO:2. A fragment of the P/CAF protein which binds to the amino acid sequence of SEQ ID NO:3 on p300 and the amino acid sequence of SEQ ID NO:9 on CBP can consist of the amino acid sequence of SEQ ID NO:4. To the extent that these fragments are specific for P/CAF, they can be used to identify and define P/CAF.

An antigenic fragment of P/CAF protein is provided. An antigenic fragment has an amino acid sequence of at least about five consecutive amino acids of a P/CAF protein amino acid sequence and binds an antibody or elicits an immune response in an animal. An antigenic fragment can be selected by applying the routine technique of epitope mapping to P/CAF protein to determine the regions of the proteins that contain epitopes reactive with antibodies or are capable of eliciting an immune response in an animal. Once the epitope is selected, an antigenic polypeptide containing the epitope can be synthesized directly, or produced recombinantly by cloning nucleic acids encoding the antigenic polypeptide in an expression system, according to standard methods.

Alternatively, an antigenic fragment of the antigen can be isolated from the whole P/CAF protein or a larger fragment of the P/CAF protein by chemical or mechanical disruption. Fragments can also be randomly chosen from a known P/CAF protein sequence and synthesized. The purified fragments thus obtained can be tested to determine their antigenicity and specificity by routine methods.

Nucleic Acids Encoding P/CAF Protein

An isolated nucleic acid that encodes a P/CAF protein is also provided. As used herein, the term "isolated" means a nucleic acid separated or substantially free from at least some of the other components of the naturally occurring organism, for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids. The isolation of nucleic acids can therefore be accomplished by techniques such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids (39). It is not contemplated that the isolated nucleic acids are necessarily totally free of all non-nucleic acid components or all other nucleic acids, but that the isolated nucleic acids are isolated to a degree of purification to be useful in clinical, diagnostic, experimental, or other procedures such as, for example, gel electrophoresis, Southern, Northern or dot blot hybridization, or polymerase chain reaction (PCR).

A skilled artisan in the field will readily appreciate that there are a multitude of procedures which may be used to isolate the nucleic acids prior to their use in other procedures. These include, but are not limited to, lysis of the cell followed by gel filtration or anion exchange chromatography, binding DNA to silica in the form of glass beads, filters or diatoms in the presence of high concentrations of chaotropic salts, or ethanol precipitation of the nucleic acids.

The nucleic acids of the present invention can include positive and negative strand RNA as well as DNA and can include genomic and subgenomic nucleic acids found in the naturally occurring organism. The nucleic acids contemplated by the present invention include double stranded and single stranded DNA of the genome, complementary positive stranded cRNA and mRNA, and complementary cDNA produced therefrom and any nucleic acid which can selectively or specifically hybridize to the isolated nucleic acids provided herein. Stringent conditions (further described below) are used to distinguish selectively or specifically hybridizing nucleic acids from non-selectively and non-specifically hybridizing nucleic acids.

An isolated nucleic acid that encodes a P/CAF protein can be species-specific (i.e., does not encode the P/CAF protein of other species and does not occur in other species). Examples of the nucleic acids contemplated herein include the nucleic acid of SEQ ID NO:10 as well as the nucleic acids that encode each of the P/CAF proteins or fragments thereof described herein. P/CAF proteins and protein fragments can be routinely obtained as described herein and their structure (sequence) determined by routine means including the methods as used herein.

P/CAF protein-encoding nucleic acids can be isolated from an organism in which they are normally found (e.g., humans), using any of the routine techniques. For example, a genomic DNA or cDNA library can be constructed and screened for the presence of the nucleic acid of interest using one of the present P/CAF protein-encoding nucleic acids as a probe. Methods of constructing and screening such libraries are well known in the art and kits for performing the construction and screening steps are

commercially available (for example, Stratagene Cloning Systems, La Jolla, CA). Once isolated, the nucleic acid can be directly cloned into an appropriate vector, or if necessary, be modified to facilitate the subsequent cloning steps. Such modification steps are routine, an example of which is the addition of oligonucleotide linkers, which contain restriction sites, to the termini of the nucleic acid (See, for example, ref. 39).

P/CAF protein-encoding nucleic acids can also be synthesized. For example, a method of obtaining a DNA molecule encoding a specific P/CAF protein is to synthesize a recombinant DNA molecule which encodes the P/CAF protein. For example, nucleic acid synthesis procedures are routine in the art and oligonucleotides coding for a particular protein region are readily obtainable through automated DNA synthesis. A nucleic acid for one strand of a double-stranded molecule can be synthesized and hybridized to its complementary strand. One can design these oligonucleotides such that the resulting double-stranded molecule has either internal restriction sites or appropriate 5' or 3' overhangs at the termini for cloning into an appropriate vector.

Oligonucleotides complementary to or identical with the P/CAF protein-encoding nucleic acid sequence can be synthesized as primers for amplification reactions, such as PCR, or as probes to detect P/CAF protein encoding nucleic acids by various hybridization protocols (e.g., Northern blot; Southern blot; dot blot, colony screening, etc.).

Double-stranded molecules coding for relatively large proteins can readily be synthesized by first constructing several different double-stranded molecules that code for particular regions of the protein, followed by ligating these DNA molecules together. For example, Cunningham, et al. (40), have constructed a synthetic gene encoding the human growth hormone by first constructing overlapping and complementary synthetic oligonucleotides and ligating these fragments together. See also, Ferretti, et al. (41), wherein synthesis of a 1057 base pair synthetic bovine rhodopsin gene from synthetic oligonucleotides is disclosed.

By constructing a P/CAF protein-encoding nucleic acid in this manner, one skilled in the art can readily obtain any particular P/CAF protein with modifications at any particular position or positions. See also, U.S. Patent No. 5,503,995 which describes an enzyme template reaction method of making synthetic genes. Techniques such as this are routine in the art and are well documented. DNA encoding the P/CAF protein or P/CAF protein fragments can then be expressed *in vivo* or *in vitro*.

The nucleic acid encoding the P/CAF protein can be any nucleic acid that functionally encodes the P/CAF protein. To functionally encode the protein (i.e., allow the nucleic acid to be expressed), the nucleic acid can include, but is not limited to, expression control sequences, such as an origin of replication, a promoter, regions upstream or downstream of the promoter, such as enhancers that may regulate the transcriptional activity of the promoter, appropriate restriction sites to facilitate cloning of inserts adjacent to the promoter, antibiotic resistance genes or other markers which can serve to select for cells containing the vector or the vector containing the insert, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites and transcription termination sequences as well as any other sequence which may facilitate the expression of the inserted nucleic acid.

Preferred expression control sequences are promoters derived from metallothionine genes, actin genes, immunoglobulin genes, CMV, SV40, adenovirus, bovine papilloma virus, etc. A nucleic acid encoding a P/CAF protein can readily be determined based upon the genetic code for the amino acid sequence of the P/CAF protein and many nucleic acid sequences will encode a P/CAF protein. Modifications in the nucleic acid sequence encoding the P/CAF protein are also contemplated. Modifications that can be useful are modifications to the sequences controlling expression of the P/CAF protein to make production of P/CAF protein inducible or repressible as controlled by the appropriate inducer or repressor. Such means are standard in the art (*see, e.g.*, ref. 39). The nucleic acids can be generated by means standard in the art, such as by recombinant nucleic acid techniques, as exemplified in the examples herein, and by synthetic nucleic acid synthesis or *in vitro* enzymatic synthesis.

After a nucleic acid encoding a particular P/CAF protein of interest, or a region of that nucleic acid, is constructed, modified, or isolated, that nucleic acid can then be cloned into an appropriate vector, which can direct the *in vivo* or *in vitro* synthesis of that wild-type and/or modified P/CAF protein. The vector is contemplated to have the necessary functional elements that direct and regulate transcription of the inserted nucleic acid, as described above. The vector containing the P/CAF nucleic acid or nucleic acid fragment can be in a host (e.g., cell or transgenic animal) for expressing the nucleic acid. The P/CAF protein or fragment thereof can thus be produced in a host system containing the expression vector and its functional activity as described herein can be demonstrated according to methods well known in the art.

There are numerous *E. coli* (*Escherichia coli*) expression vectors known to one of ordinary skill in the art useful for the expression of proteins. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteria, such as *Salmonella, Serratia*, as well as various *Pseudomonas* species. These prokaryotic hosts can support expression vectors which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (Trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence and have ribosome binding site sequences, for example, for initiating and completing transcription and translation. If necessary, an amino terminal methionine can be provided by insertion of a Met codon 5' and in-frame with the gene sequence. Also, the carboxy-terminal extension of the protein can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression can be used. There are several advantages to yeast expression systems. First, evidence exists that proteins produced in yeast secretion systems exhibit correct disulfide pairing. Second, post-translational glycosylation is efficiently carried out by yeast secretory systems. The *Saccharomyces cerevisiae* pre-pro-alpha-factor leader region (encoded by the *MFα-1* gene) is routinely used to direct

protein secretion from yeast (42). The leader region of pre-pro-alpha-factor contains a signal peptide and a pro-segment which includes a recognition sequence for a yeast protease encoded by the *KEX2* gene. This enzyme cleaves the precursor protein on the carboxyl side of a Lys-Arg dipeptide cleavage-signal sequence. The polypeptide coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region. This construct is then put under the control of a strong transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The protein coding sequence is followed by a translation termination codon which is followed by transcription termination signals. Alternatively, the polypeptide encoding sequence of interest can be fused to a second protein coding sequence, such as Sj26 or β-galactosidase, used to facilitate purification of the resultant fusion protein by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion protein is applicable to constructs used for expression in yeast.

Efficient post-translational glycosylation and expression of recombinant proteins can also be achieved in *Baculovirus* expression systems in insect cells.

Mammalian cells permit the expression of proteins in an environment that favors important post-translational modifications such as folding and cysteine pairing, addition of complex carbohydrate structures and secretion of active protein. Vectors useful for the expression of proteins in mammalian cells are characterized by insertion of the protein encoding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or methotrexate resistance for use as selectable markers. For example, the antigen and immunoreactive fragment coding sequence can be introduced into a Chinese hamster ovary (CHO) cell line using a methotrexate resistance-encoding vector. Presence of the vector RNA in transformed cells can be confirmed by Northern blot analysis and production of a cDNA or opposite strand RNA corresponding to the protein encoding sequence can be confirmed by Southern and Northern blot analysis, respectively. A number of other suitable host cell lines capable of secreting intact proteins have been developed in the art and include the CHO cell lines, HeLa cells, myeloma cell lines, Jurkat cells, and the like.

Expression vectors for these cells can include expression control sequences, as described above. The vectors containing the nucleic acid sequences of interest can be transferred into the host cell by well-known methods, which vary depending on the type of cell host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cell hosts.

Alternative vectors for the expression of protein in mammalian cells, similar to those developed for the expression of human gamma-interferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface antigen, protease Nexin I, and eosinophil major basic protein, can be employed. Further, the vector can include CMV promoter sequences and a polyadenylation signal available for expression of inserted nucleic acid in mammalian cells (such as COS7).

The nucleic acid sequences can be expressed in hosts after the sequences have been positioned to ensure the functioning of an expression control sequence. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors can contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired nucleic acid sequences (*see*, e.g., U.S. Patent 4,704,362).

The nucleic acids produced as described above can also be expressed in a host which is a non-human animal to create a transgenic animal, containing, in a germ or somatic cell, a nucleic acid comprising the coding sequence for all or a portion of the P/CAF protein, as well as all of the other regulatory elements required for expression of the P/CAF protein-encoding sequence. The animal will express the P/CAF gene or portion thereof to produce the P/CAF protein or protein fragment and such expression can be detected by determination of a particular phenotype unique to the transgenic animal expressing the transferred nucleic acid.

The nucleic acid can be the nucleic acid of SEQ ID NO:10, a nucleic acid having a nucleotide sequence which encodes the P/CAF protein, a nucleic acid having a nucleotide sequence which encodes the protein of SEQ ID NO:1, as well as the nucleic acids that encode the proteins comprising the fragments of SEQ ID NOS:2 and 4.

The nucleic acids of the invention can contain substitutions or deletions which provide a particular phenotype of interest. For example, various deletions or base substitutions can be introduced into the nucleic acid encoding the P/CAF protein for the purpose of studying the effects of these particular deletions or substitutions on the transcription modulation activity of the P/CAF protein. These effects can be monitored by observation of such characteristics as growth and development of the animal, the ability to develop tumors, survival rates and the like. The gene construct introduced into the animal cells to produce the transgenic animal can contain any of the regulatory elements described above to modulate expression of the foreign genes. As used herein, the term "phenotype" includes morphology, biochemical profiles, changes in tumor formation and other parameters that are affected by the presence of the P/CAF protein.

The transgenic animals of the invention can also be used in a method for determining the effectiveness of administering a nucleic acid encoding a functional P/CAF protein to a subject in need of a functional P/CAF protein. First, a nucleic acid encoding a nonfunctional P/CAF protein can be introduced into the animal's cells and expressed to yield a characteristic phenotype. Then, using standard gene therapy techniques, a nucleic acid encoding a functional P/CAF protein can be introduced into the animal's cells and the effects on the animal's phenotypic characteristics can be determined.

Having provided and taught how to obtain a nucleic acid that encodes a P/CAF protein, an isolated nucleic acid that encodes a fragment of P/CAF protein is also provided. The nucleic acid encoding the fragment can be obtained using any of the methods applicable to the nucleic acid encoding the entire P/CAF protein. The nucleic acid fragment can encode a species-specific P/CAF protein fragment (e.g., found in the

P/CAF protein of humans, but not in the P/CAF proteins of other species). Nucleic acids encoding species-specific fragments of P/CAF protein are themselves species-specific or allele-specific fragments of the P/CAF gene.

Examples of fragments of a nucleic acid encoding a fragment of the P/CAF protein can include the nucleic acid sequences which encode the amino acid sequences of the fragments of SEQ ID NOS:2 or 4. The same routine computer analyses used to select these examples of fragments can be routinely used to obtain others. Fragments of P/CAF-encoding nucleic acids can be primers for PCR or probes, which can be species-specific, gene-specific or allele-specific. P/CAF-encoding nucleic acid fragments can encode antigenic or immunogenic fragments of P/CAF protein that can be used in therapeutic assays or screening protocols. P/CAF gene fragments can encode fragments of P/CAF protein having histone acetylase activity and/or p300/CBP binding activity as described above, as well as other uses that may become apparent.

An isolated nucleic acid of at least ten nucleotides that selectively hybridizes with the nucleic acid of SEQ ID NO:10 under selected conditions is provided. For example, the conditions can be PCR amplification conditions and the hybridizing nucleic acid can be a primer consisting of a specific fragment of the reference sequence or a nearly identical nucleic acid that hybridizes only to the exemplified P/CAF-encoding nucleic acid or allelic variants thereof.

The invention provides an isolated nucleic acid that selectively hybridizes with the P/CAF-encoding nucleic acid sequence of SEQ ID NO:10 under stringent conditions. The hybridizing nucleic acid can be a probe that hybridizes only to the exemplified P/CAF-encoding nucleic acid sequence. Thus, the hybridizing nucleic acid can be a naturally occurring species-specific allelic variant of the exemplified P/CAF gene. The hybridizing nucleic acid can also include insubstantial base substitutions that do not prevent hybridization under the stated stringent conditions or affect either the function of the encoded protein, the way the protein accomplishes that function (e.g., its

secondary structure) or the ultimate result of the protein's activity. The means for determining these parameters are well known.

As used herein to describe nucleic acids, the term "selectively hybridizes" excludes the occasional randomly hybridizing nucleic acids as well as nucleic acids that encode other known homologs of the P/CAF protein. The selectively hybridizing nucleic acids of the invention can have at least 70%, 73%, 78%, 80%, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementarity with the segment and strand of the sequence to which it hybridizes. This list is not intended to exclude percent complementarity values between these values. The nucleic acids can be at least 10, 15, 16, 17, 18, 20, 21, 23, 24, 25, 30, 35, 40, 50, 100, 150, 200, 300, 500, 550, 750, 900, 950, or 1000 nucleotides in length or any intervening length, depending on whether the nucleic acid is to be used as a primer, probe or for protein expression. The hybridizing nucleic acid can comprise a region of at least ten nucleotides (up to full length) that is completely complementary to a unique region of the nucleic acid to which it hybridizes.

The nucleic acid can be an alternative coding sequence for the P/CAF protein, or can be used as a probe or primer for detecting the presence of or obtaining the P/CAF protein. If used as primers, the invention provides compositions including at least two nucleic acids which selectively hybridize with different regions of the nucleic acid so as to amplify a desired region. Depending on the length of the probe or primer, it can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions.

For example, for the purpose of obtaining or determining the presence of a nucleic acid encoding the P/CAF protein, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (P/CAF DNA in a sample) should be at least enough to exclude hybridization with a nucleic acid from another species. The invention provides examples of these nucleic acids of P/CAF, so that the degree of complementarity required to distinguish selectively

hybridizing from nonselectively hybridizing nucleic acids under stringent conditions can be clearly determined for each nucleic acid. It should also be clear that the hybridizing nucleic acids of the invention will not hybridize with nucleic acids encoding unrelated proteins (hybridization is selective) under stringent conditions.

"Stringent conditions" refers to the washing conditions used in a hybridization protocol. In general, the washing conditions should be a combination of temperature and salt concentration chosen so that the denaturation temperature is approximately 5-20°C below the calculated T_m of the nucleic acid hybrid under study. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to the probe or protein encoding nucleic acid of interest and then washed under conditions of different stringencies. For example, the nucleic acid sequence of SEQ ID NO:10 was used as a specific radiolabeled probe for the detection of messenger RNA transcribed from the P/CAF gene by performing hybridizations under stringent conditions. The T_m of such an oligonucleotide can be estimated by allowing 2°C for each A or T nucleotide, and 4°C for each G or C. For example, an 18 nucleotide probe of 50% G+C would, therefore, have an approximate T_m of 54°C.

The invention provides an isolated nucleic acid that selectively hybridizes with the P/CAF gene shown in the sequence set forth as SEQ ID NO:10 under stringent conditions. The invention further provides an isolated nucleic acid complementary to the nucleotide sequence set forth in SEQ ID NO:10.

Antibodies to the P/CAF protein

A purified antibody and an antiserum containing polyclonal antibodies that specifically bind the P/CAF protein or antigenic fragment are also provided. The term "bind" means the well understood antigen/antibody binding as well as other nonrandom association with an antigen. "Specifically bind" as used herein describes an antibody or other ligand that does not cross react substantially with any antigen other than the one specified, in this case, an antigen of the P/CAF protein. Antibodies can be made as

described in Harlow and Lane (33). Briefly, purified P/CAF protein or an antigenic fragment thereof can be injected into an animal in an amount and in intervals sufficient to elicit a humoral immune response. Serum polyclonal antibodies can be purified directly, or spleen cells from the animal can be fused with an immortal cell line and screened for monoclonal antibody secretion, according to procedures well known in the art. Purified monospecific polyclonal antibodies that specifically bind the P/CAF antigen are also within the scope of the present invention. The antibodies of the present invention can bind the protein of claim 1, the protein of claim 2, the protein of claim 3 and/or the protein of claim 4, as well as any other proteins of the present invention.

A ligand that specifically binds the antigen is also contemplated. The ligand can be a fragment of an antibody, such as , for example, an Fab fragment which retains P/CAF binding activity, or a smaller molecule designed to bind an epitope of the P/CAF antigen. The antibody or ligand can be bound to a substrate or labeled with a detectable moiety or both bound and labeled. The detectable moieties contemplated within the compositions of the present invention include those listed above in the description of the diagnostic methods, including fluorescent, enzymatic and radioactive markers.

The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or therapeutic compound or both bound and conjugated. Such conjugation techniques are well known in the art. For example, conjugation of fluorescent, radioactive or enzymatic moieties can be performed as described in the art (33,43). The detectable moieties contemplated in the present invention can include fluorescent, radioactive and enzymatic markers and the like. Therapeutic drugs contemplated with the present invention can include cytotoxic moieties such as ricin A chain, diphtheria toxin, pseudomonas exotoxin and other chemotherapeutic compounds.

It is well understood by one of skill in the art that all of the above discussion regarding antibodies to P/CAF can also be applied with regard to production, characterization and use of antibodies which bind the p300/CBP protein or any of the DNA-binding transcription factors of this invention.

Measuring the P/CAF protein in a sample

The present invention also provides a method for determining the presence and thus the amount of P/CAF protein in a biological sample. As used herein, a biological sample includes any tissue or cell which would contain the P/CAF protein. Examples of cells include tissues taken from surgical biopsies, isolated from a body fluid or prepared in an *in vitro* tissue culture environment.

One example of determining the amount of P/CAF in a biological sample can comprise contacting the biological sample with a polypeptide comprising the amino acid sequence of SEQ ID NO:3 under conditions whereby a P/CAF/p300 complex can be formed; and determining the amount of the P/CAF/p300 complex, the amount of the complex indicating the amount of P/CAF in the sample. Determination of the amount of P/CAF/p300 complex can be accomplished through techniques standard in the art. For example, the complex may be precipitated out of a solution and detected by the addition of a detectable moiety conjugated to the p300 protein or by the detection of an antibody which binds p300 or the P/CAF protein, as taught in the Examples herein. Antibodies which bind p300 or the P/CAF protein can be either monoclonal or polyclonal antibodies and can be obtained as described herein. Detection of P/CAF/p300 complexes by the detection of the binding of antibodies reactive with p300 or the P/CAF protein can be accomplished using various immunoassays as are available in the art, as described below.

Alternatively, determination of the amount of P/CAF in a biological sample can comprise contacting the biological sample with a polypeptide comprising the amino acid sequence of SEQ ID NO:9 under conditions whereby a P/CAF/CBP complex can be formed; and determining the amount of the P/CAF/CBP complex, the amount of the complex indicating the amount of P/CAF in the sample. Determination of the amount of P/CAF/CBP complex can be accomplished through techniques standard in the art. For example, the complex may be precipitated out of a solution and detected by the addition of a detectable moiety conjugated to the CBP protein or by the detection of an antibody which binds either CBP or the P/CAF protein, as taught in the Examples

herein. Antibodies which bind CBP or the P/CAF protein can be either monoclonal or polyclonal antibodies and can be obtained as described herein. Detection of P/CAF/CBP complexes by the detection of the binding of antibodies reactive with CBP or the P/CAF protein can be accomplished using various immunoassays as are available in the art, as described below.

Another example of determining the amount of P/CAF in a biological sample comprises contacting the biological sample with an antibody which specifically binds P/CAF under conditions whereby a P/CAF/ antibody complex can be formed and determining the amount of the P/CAF/antibody complex, the amount of the complex indicating the amount of P/CAF in the sample. Antibodies which bind P/CAF can be either monoclonal or polyclonal antibodies and can be obtained as described herein. Determination of P/CAF/antibody complexes can be accomplished using various immunoassays as are available in the art, as described below.

Immunoassays such as immunofluorescence assays, radioimmunoassays (RIA), immunoblotting and enzyme linked immunosorbent assays (ELISA) can be readily adapted for detection and measurement of P/CAF in a biological sample. Both polyclonal and monoclonal antibodies can be used in the assays. Available immunoassays are well known in the art and are extensively described in the patent scientific literature. See, for example, U.S. Patent Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876.

Screening assays for P/CAF

The present invention also provides a bioassay for screening substances for the ability to inhibit the histone acetyltransferase activity of P/CAF comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance under conditions whereby histone acetylation by P/CAF can occur; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the

amount of histone acetylation by P/CAF in the absence of the substance, a decreased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit the histone acetyltransferase activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to inhibit the histone acetyltransferase activity of P/CAF can be added to this system and assayed for inhibiting ability.

The present invention also provides a bioassay for screening substances for the ability to inhibit the transcription modulating activity of P/CAF, comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance under conditions whereby histone acetylation by P/CAF can occur; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit the transcription modulating activity and cell cycle progression suppressing activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to inhibit the transcription modulating activity of P/CAF by interfering with the histone acetyltransferase activity of P/CAF can be added to this system and assayed for inhibiting ability.

Also provided in the present invention is a bioassay for screening substances for the ability to inhibit the binding of p300 to P/CAF, comprising contacting a system in which the binding of p300 to P/CAF can be determined, with the substance under conditions whereby the binding of p300 and P/CAF can occur; determining the amount of p300 binding to P/CAF in the presence of the substance; and comparing the amount of p300 binding to P/CAF in the presence of the substance with the amount of p300 binding to P/CAF in the absence of the substance, a decreased amount of p300 binding to P/CAF in the presence of the substance indicating a substance that can inhibit the binding of p300 to P/CAF. The binding of p300 to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the p300 protein comprising the amino acid sequence of SEQ ID NO:3 and P/CAF. Alternatively, the system can comprise a cell extract produced from cells producing both p300 and P/CAF. Determination of the binding of p300 to P/CAF can be carried out as taught herein.

Additionally provided in the present invention is a bioassay for screening substances for the ability to inhibit the binding of CBP to P/CAF, comprising contacting a system in which the binding of CBP to P/CAF can be determined, with the substance under conditions whereby the binding of CBP to P/CAF can occur; determining the amount of CBP binding to P/CAF in the presence of the substance; and comparing the amount of CBP binding to P/CAF in the presence of the substance with the amount of CBP binding to P/CAF in the absence of the substance, a decreased amount of CBP binding to P/CAF in the presence of the substance indicating a substance that can inhibit the binding of CBP to P/CAF. The binding of CBP to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the CBP protein comprising the amino acid sequence of SEQ ID NO:9 and P/CAF. Alternatively, the system can comprise a cell extract produced from cells producing both CBP and P/CAF. Determination of the binding of CBP to P/CAF can be carried out as taught herein.

The present invention further contemplates a bioassay for screening substances for the ability to stimulate the histone acetyltransferase activity of P/CAF comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, an increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can stimulate the histone acetyltransferase activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to stimulate the histone acetyltransferase activity of P/CAF can be added to this system and assayed for stimulating ability.

The present invention further contemplates a bioassay for screening substances for the ability to stimulate the transcription modulating activity of P/CAF comprising contacting a system, in which histone acetylation by P/CAF can be determined, with the substance; determining the amount of histone acetylation by P/CAF in the presence of the substance; and comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, an increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can stimulate the transcription modulating activity of P/CAF. The acetylation of histones by P/CAF can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P/CAF protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples.

Thus, the compound to be tested for the ability to stimulate the transcription modulating activity of P/CAF by increasing the histone acetyltransferase activity of P/CAF can be added to this system and assayed for stimulating ability.

The present invention further provides a bioassay for screening substances for the ability to stimulate binding of p300 to P/CAF, comprising contacting a system in which the binding of p300 to P/CAF can be determined, with the substance under conditions whereby the binding of p300 to P/CAF can occur; determining the amount of p300 binding to P/CAF in the presence of the substance; and comparing the amount of p300 binding to P/CAF in the presence of the substance with the amount of p300 binding to P/CAF in the absence of the substance, an increased amount of p300 binding to P/CAF in the presence of the substance indicating a substance that can stimulate the binding of p300 to P/CAF. The binding of p300 to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the p300 protein comprising the amino acid sequence of SEQ ID NO:3 and P/CAF. Alternatively, the system can comprise a cell extract produced from cells producing both p300 and P/CAF. Determination of the binding of p300 to P/CAF can be carried out as taught herein.

Additionally provided in the present invention is a bioassay for screening substances for the ability to stimulate the binding of CBP to P/CAF, comprising contacting a system in which the binding of CBP to P/CAF can be determined, with the substance under conditions whereby the binding of CBP to P/CAF can occur; determining the amount of CBP binding to P/CAF in the presence of the substance; and comparing the amount of CBP binding to P/CAF in the presence of the substance with the amount of CBP binding to P/CAF in the absence of the substance, an increased amount of CBP binding to P/CAF in the presence of the substance indicating a substance that can stimulate the binding of CBP to P/CAF. The binding of CBP to P/CAF can be determined in a system, for example, which can include a cell free reaction mixture comprising a fragment of the CBP protein comprising the amino acid sequence of SEQ ID NO:9 and P/CAF. Alternatively, the system can comprise a cell

extract produced from cells producing both CBP and P/CAF. Determination of the binding of CBP to P/CAF can be carried out as taught herein.

Transcription modulating activity of P/CAF

The present invention contemplates a method for inhibiting the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity inhibiting amount of a substance in a pharmaceutically acceptable carrier. For example, the substance can be identified according to the protocols provided herein as one that can inhibit the transcription modulating activity of P/CAF by preventing the binding of P/CAF to p300/CBP or by inhibiting the histone acetyltransferase activity of P/CAF as well as by any other inhibitory mechanism as identified by the protocols provided herein. Inhibition of the transcription modulating activity of P/CAF in a subject is desirable, for example, to inhibit HIV TAT-mediated transcription and therefore, the method of the present invention can be used to treat HIV-infected subjects.

The substance can be in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the substance, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject.

The transcription modulating activity and/or histone acetyltransferase activity of P/CAF can be inhibited in a subject by administering to the subject a substance which binds p300/CBP at the P/CAF binding site or a substance which binds the P/CAF protein at the p300/CBP binding site, the ultimate result being that P/CAF and p300/CBP do not bind with one another and P/CAF cannot exert its transcription modulating and/or histone acetyltransferase effect. The substance can be a protein, such as an antibody which binds the P/CAF protein binding site at or near the p300/CBP

binding site, thereby preventing its binding or an antibody which binds the p300/CBP protein at or near the P/CAF binding site, thereby preventing its binding. The substance can also bind the histone acetyltransferase site on P/CAF or at the acetylation site on the histone, thereby preventing acetylation by P/CAF.

The substance which binds p300/CBP, the P/CAF protein or the histone and has the net effect of inhibiting the transcription modulating effect and or histone acetyltransferase activity of P/CAF in the cell can be delivered to a cell in the subject by mechanisms well known in the art.

Alternatively, a nucleic acid encoding a protein which binds either to p300/CBP or the P/CAF protein and has the net effect of inhibiting the transcription modulating effect and/or histone acetyltransferase activity of P/CAF in the cell can be delivered to a cell in the subject by gene transduction mechanisms well known in the art. For example, nucleic acid can be introduced by liposomes as well as via retroviral or adeno-associated viral vectors, as described below.

The substance which inhibits the transcription modulating effect and/or histone acetyltransferase activity of P/CAF can be an antisense RNA or an antisense DNA which binds the RNA or DNA of P/CAF, thereby preventing translation or transcription of the RNA or DNA encoding P/CAF and having the net effect of inhibiting the transcription modulating effect and/or histone acetyltransferase activity of P/CAF by inhibiting P/CAF production. The antisense RNA of the present invention can be generated from the nucleic acid of SEQ ID NO:14 (human) or SEQ ID NO:15 (mouse). Furthermore, the antisense DNA can be a phosphorothioate oligodeoxyribonucleotide having the nucleotide sequence of SEQ ID NO:16 (human) or of SEQ ID NO:17 (mouse). The mouse antisense RNA can be used to inhibit the activity of mouse P/CAF, having the nucleotide sequence of SEQ ID NO:18 and the amino acid sequence of SEQ ID NO:8. The present invention also contemplates an antisense nucleic acid sequence which can bind the DNA or RNA of any of the transcription factors or other proteins now known or later identified to bind P/CAF, thereby inhibiting expression of the gene products of

these proteins and having the net effect of inhibiting the transcription modulating effect and/or histone acetyltransferase activity of P/CAF.

The antisense nucleic acid can comprise a typical nucleic acid, but the antisense nucleic acid can also be a modified nucleic acid or a derivative of a nucleic acid such as a phosphorothioate analogue of a nucleic acid. The composition can comprise, for example, an antisense RNA that specifically binds an RNA encoded by the gene encoding the serum protein. Antisense RNAs can be synthesized and used by standard methods (62).

Antisense RNA can inhibit gene expression by forming an RNA/RNA duplex between the antisense RNA and the RNA transcribed from the target gene. The precise mechanism by which this duplex formation decreases the production of the protein encoded by the endogenous gene probably involves binding of complementary regions of the normal sense mRNA and the antisense RNA strand with duplex formation in a manner that blocks RNA processing and translation. Alternative mechanisms include the formation of a triplex between the antisense RNA and duplex DNA or the formation of an DNA-RNA duplex with subsequent degradation of DNA-RNA hybrids by RNAse H. Furthermore, an antigene effect can result from certain DNA-based oligonucleotides via triple-helix formation between the oligomer and double-stranded DNA which results in the repression of gene transcription. Regardless of the specific molecular mechanism, the present invention results in inhibition of expression of the P/CAF gene by the introduced and replicated DNA resulting in inhibition of the transcription modulating and/or histone acetyltransferase activity of P/CAF, by a reduction in the expression of the nucleic acid to which the antisense nucleic acid is hybridized, and therefore a reduction of the gene product from the targeted gene.

The antisense nucleic acid may be obtained by any number of techniques known to one skilled in the art. One method of constructing an antisense nucleic acid is to synthesize a recombinant antisense DNA molecule. For example, oligonucleotide synthesis procedures are routine in the art and oligonucleotides coding for a particular

protein or regulatory region are readily obtainable through automated DNA synthesis. A nucleic acid for one strand of a double-stranded molecule can be synthesized and hybridized to its complementary strand. One can design these oligonucleotides such that the resulting double-stranded molecule has either internal restriction sites or appropriate 5' or 3' overhangs at the termini for cloning into an appropriate vector. Double-stranded molecules coding for relatively large proteins or regulatory regions can be synthesized by first constructing several different double-stranded molecules that code for particular regions of the protein or regulatory region, followed by ligating these DNA molecules together. Once the appropriate DNA molecule is synthesized, this DNA can be cloned downstream of a promoter in an antisense orientation. Techniques such as this are routine in the art and are well documented.

An example of another method of obtaining an antisense nucleic acid is to isolate that nucleic acid from the organism in which it is found and clone it in an antisense orientation. For example, a DNA or cDNA library can be constructed and screened for the presence of the nucleic acid of interest. Methods of constructing and screening such libraries are well known in the art and kits for performing the construction and screening steps are commercially available (for example, Stratagene Cloning Systems, La Jolla, CA). Once isolated, the nucleic acid can be directly cloned into an appropriate vector in an antisense orientation, or if necessary, be modified to facilitate the subsequent cloning steps. Such modification steps are routine, an example of which is the addition of oligonucleotide linkers which contain restriction sites to the termini of the nucleic acid. General methods are set forth in Sambrook *et al.* (39).

The DNA that is introduced into the cell is in an expression orientation that is antisense to a corresponding endogenous DNA or RNA of the cells. For example, where an endogenous DNA comprises a gene which encodes for a particular protein, the introduced DNA is in an expression orientation opposite the expression of the endogenous DNA; that is the DNA operatively linked to a promoter is in an antisense expression orientation relative to the corresponding endogenous gene. The introduced DNA may be homologous to the entire transcribed gene or homologous to only part of

the transcribed gene. Alternatively, the sequence of the introduced DNA may be divergent to that of the endogenous DNA but only divergent to the extent that hybridization of the nucleic acids occurs, thereby preventing transcription. One skilled in the art can determine the maximum extent of this divergence by routine screening of antisense DNAs corresponding to an endogenous DNA of the cell. In this manner, one skilled in the art can readily determine which fragments, or alternatively the extent of homology of the fragments or the entire gene that is necessary to inhibit gene expression.

The antisense nucleic acids of the present invention can be made according to protocols standard in the art, as well as described in the Examples provided herein. The antisense nucleic acids can be administered to a subject according to the gene transduction protocols standard in the art, as described below.

The present invention also contemplates a method for stimulating the transcription modulating activity and/or histone acetyltransferase activity of P/CAF in a subject comprising administering to the subject a substance, in a pharmaceutically acceptable carrier, determined according to the methods taught herein, to have a stimulatory affect on the transcription modulating and/or histone acetyltransferase activity of P/CAF. The substance can be one which has been identified, according to the protocols provided herein, to stimulate histone acetyltransferase activity in P/CAF or promote binding of P/CAF to p300/CBP. The stimulation of the transcription modulation activity and/or histone acetyltransferase activity of P/CAF in a subject is desirable, for example, to activate tumor suppressor p53 (which promotes apoptosis) or to activate the muscle differentiation factor, MyoD. Thus, the method of the present invention can be employed to treat cancer and to promote muscle differentiation in conditions where muscle differentiation is desired. The substance can be delivered to a cell in the subject by mechanisms well known in the art.

Further contemplated in the present invention is a method for promoting binding of P/CAF to p300/CBP in a subject, comprising administering to the subject a substance

identified by the methods provided herein to promote binding of P/CAF to either p300 or CBP.

Additionally, a nucleic acid encoding a protein which stimulates the transcription modulating activity and/or histone acetyltransferase activity of P/CAF can be delivered to a cell in the subject by gene transduction mechanisms, as described below.

Also provided in the present invention is a method of inhibiting the cell cycle progression inducing effect of an oncoprotein which binds p300/CBP in a subject comprising transducing the cells of the subject with a vector comprising a nucleic acid encoding the P/CAF protein; inducing expression of the nucleic acid in the cell to produce the P/CAF in an amount which will allow the P/CAF gene product to replace the oncoprotein bound to p300/CBP, whereby the replacement of the oncoprotein bound to p300/CBP by the P/CAF gene product inhibits the cell cycle progression inducing effect of the oncoprotein. The oncoprotein which binds p300/CBP in the cell can be the adenovirus E1A oncoprotein.

A method for providing a functional P/CAF protein to a subject in need of the functional P/CAF protein is also provided, comprising transducing the cells of the subject with a vector comprising a nucleic acid encoding the P/CAF protein and inducing expression of the nucleic acid to produce the functional P/CAF protein in the cell, thereby providing the functional P/CAF protein to the subject. The transduction of the vector nucleic acid into the subject's cells can be carried out according to standard gene therapy protocols well known in the art (see, for example, U.S. Patent No. 5,339,346).

Screening assays for p300/CBP

The present invention also provides a bioassay for screening substances for the ability to inhibit the histone acetyltransferase activity of p300/CBP comprising contacting a system, in which histone acetylation by p300/CBP can be determined, with the substance under conditions whereby histone acetylation by p300/CBP can occur;

determining the amount of histone acetylation by p300/CBP in the presence of the substance; and comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, a decreased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can inhibit the histone acetyltransferase activity of p300/CBP. The acetylation of histones by p300/CBP can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the P300/CBP protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to inhibit the histone acetyltransferase activity of p300/CBP can be added to this system and assayed for acetyltransferase inhibiting ability.

Also provided in the present invention is a bioassay for screening substances for the ability to inhibit the binding of a transcriptional factor to p300/CBP, comprising contacting a system in which the binding of a transcriptional factor to p300/CBP can be determined, with the substance under conditions whereby the binding of the transcriptional factor and p300/CBP can occur; determining the amount of transcriptional factor binding to p300/CBP in the presence of the substance; and comparing the amount of transcriptional factor binding to p300/CBP in the presence of the substance with the amount of transcriptional factor binding to p300/CBP in the absence of the substance, a decreased amount of transcriptional factor binding to p300/CBP in the presence of the substance indicating a substance that can inhibit the binding of a transcriptional factor to p300/CBP. The binding of a transcriptional factor to p300/CBP can be determined in a system, for example, which can include a cell free reaction mixture comprising a transcriptional factor which binds p300/CBP and p300/CBP. Alternatively, the system can comprise a cell extract produced from cells producing both a transcriptional factor which binds p300/CBP and p300/CBP. The transcriptional factor which binds p300/CBP can be selected from, but is not limited to

the group consisting of nuclear hormone receptors, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YYI, Sap-1a, c-Fos, MyoD and SRC-1, as well as any other transcriptional factor now known or later identified to bind p300/CBP. The screening assay of the present invention can also be used to identify substances which inhibit the binding of p300/CBP to other components to which it is known to bind, for example, P/CAF, $pp90_{RSK}$, TFIIB, E1A, SV40 large T antigen, as well as any other substances now known or later identified to bind p300/CBP. Determination of the binding of a transcriptional factor or other substance to p300/CBP can be carried out as taught in the Examples herein as well as by protocols described in the literature.

The present invention further contemplates a bioassay for screening substances for the ability to stimulate the histone acetyltransferase activity of p300/CBP comprising contacting a system, in which histone acetylation by p300/CBP can be determined, with the substance; determining the amount of histone acetylation by p300/CBP in the presence of the substance; and comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, an increased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can stimulate the histone acetyltransferase activity of p300/CBP. The acetylation of histones by p300/CBP can be determined in a system including, for example, either core histones (histones H2A, H2B, H3 and H4) or the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) as substrates, the p300/CBP protein and radiolabeled acetyl-CoA (e.g., [1-^{14}C]acetyl CoA). The presence of acetylated histones can be detected by autoradiography after separation by SDS-PAGE as described herein in the Examples. Thus, the compound to be tested for the ability to stimulate the histone acetyltransferase activity of p300/CBP can be added to this system and assayed for stimulating ability.

The present invention further provides a bioassay for screening substances for the ability to stimulate binding of a component, which binds p300/CBP, to p300/CBP, comprising contacting a system in which the binding of the component to p300/CBP can

be determined, with the substance under conditions whereby the binding of the component to p300/CBP can occur; determining the amount of component binding to p300/CBP in the presence of the substance; and comparing the amount of component binding to p300/CBP in the presence of the substance with the amount of component binding to p300/CBP in the absence of the substance, an increased amount of component binding to p300/CBP in the presence of the substance indicating a substance that can stimulate the binding of the component to p300/CBP. The binding of the component to p300/CBP can be determined in a system, for example, which can include a cell free reaction mixture comprising the component and p300/CBP. Alternatively, the system can comprise a cell extract produced from cells producing both the component and p300/CBP. The component which binds p300/CBP can be any of the transcriptional factors or other proteins which are known or are identified in the future to bind p300/CBP, as set forth above. Determination of the binding of the component to p300/CBP can be carried out as taught in the Examples provided herein and according to protocols available in the literature.

Histone acetyltransferase activity of p300/CBP

A method for inhibiting the histone acetyltransferase activity of p300/CBP in a subject is provided in the present invention, comprising administering to the subject a histone acetyltransferase activity inhibiting amount of a substance in a pharmaceutically acceptable carrier. The mechanism of the inhibitory action of the substance can be the inhibition of the binding of a DNA-binding transcription factor, such as, for example, a nuclear hormone receptor, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YY1, Sap-1a, c-Fos, MyoD or SRC-1, to p300/CBP.

The histone acetyltransferase activity of p300/CBP can be inhibited in a subject by administering to the subject a substance which binds p300/CBP at the transcription factor binding site or a substance which binds the transcription factor protein at the p300/CBP binding site, the ultimate result being that the transcription factor and p300/CBP do not bind with one another and p300/CBP cannot acetylate histones.

The substance which binds either to the transcription factor or the p300/CBP protein and has the net effect of inhibiting the histone acetyltransferase activity of p300/CBP in the cell can be identified according to the screening methods provided herein and delivered to a cell in the subject by mechanisms well known in the art. The substance can be a protein, such as an antibody which binds the p300/CBP protein binding site at or near the DNA-binding transcription factor binding site, thereby preventing its binding or an antibody which binds the DNA-binding transcription factor at or near the p300/CBP binding site, thereby preventing its binding. The substance can also bind the histone acetyltransferase site on p300/CBP (aa 1195-1673 on p300 or aa 1174-1850 on CBP) or at the acetylation site on the histone, thereby preventing acetylation by p300/CBP.

Additionally, the substance can be a nucleic acid which can be expressed in the cell to produce a protein which inhibits the histone acetyltransferase activity of p300/CBP. For example, a nucleic acid encoding a protein which binds either to a transcription factor or the p300/CBP protein and has the net effect of inhibiting the histone acetyltransferase activity of p300/CBP in the cell can be delivered to a cell in the subject by gene transduction mechanisms well known in the art. For example, nucleic acid can be introduced by liposomes as well as via retroviral or adeno-associated viral vectors, as described below.

The substance which inhibits the histone acetyltransferase activity of p300/CBP can be an antisense RNA or an antisense DNA which binds the RNA or DNA of p300/CBP thereby preventing translation or transcription of the RNA or DNA encoding p300/CBP and having the net effect of inhibiting the histone acetyltransferase activity of P/CAF by inhibiting p300/CBP production. The antisense RNA or DNA of the present invention can be produced and introduced into cells according to the same methods as set forth above for P/CAF antisense nucleic acids.

The present invention also contemplates a method for stimulating the histone acetyltransferase activity of p300/CBP in a subject comprising administering to the

subject a histone acetyltransferase activity stimulating amount of a substance, in a pharmaceutically acceptable carrier, determined according to the methods taught herein, to have a stimulatory affect on the histone acetyltransferase activity of p300/CBP. The substance can exert a stimulatory effect by promoting the binding of a DNA-binding transcription factor of the present invention to p300/CBP. The substance can be delivered to a cell in the subject by mechanisms well known in the art. A nucleic acid encoding a protein which stimulates the transcription modulating activity of p300/CBP can be delivered to a cell in the subject by gene transduction mechanisms, as described below.

Gene transduction

In the methods described above which include gene transduction into cells (i.e., addition of exogenous DNA into cells), the nucleic acids of the present invention can be in a vector for delivering the nucleic acids to the site for expression of the P/CAF protein. The vector can be one of the commercially available preparations, such as the pGM plasmid (Promega). Vector delivery can be by liposome, using commercially available liposome preparations or newly developed liposomes having the features of the present liposomes. Additionally, vector delivery can be via a viral system, including, but not limited to, retroviral, adenoviral and adeno-associated viral systems. Other delivery methods can be adopted and routinely tested according to the methods taught herein.

The modes of administration of the liposome will vary predictably according to the disease being treated and the tissue being targeted. For example, for treating cancer in either the lung or the liver, which are both sinks for liposomes, intravenous delivery is reasonable. For other localized cancers, as well as precancerous conditions, catheterization of an artery upstream from the target organ is a preferred mode of delivery, because it avoids significant clearance of the liposome by the lung and liver. For cancerous lesions at a number of other sites (e.g., skin cancer, localized dysplasias), topical delivery is expected to be effective and may be preferred, because of its convenience.

Leukemias and other disorders involving dysregulated proliferation of certain isolatable cell populations may be more readily treated by *ex vivo* administration of the nucleic acid.

The liposomes may be administered topically, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally or the like, although intravenous or topical administration is typically preferred. The exact amount of the liposomes required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disease being treated, the particular compound used, its mode of administration and the like. Thus, it is not possible to specify an exact amount. However, an appropriate amount may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

Topical administration can be by creams, gels, suppositories and the like. *Ex vivo* (extracorporeal) delivery can be as typically used in other contexts.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLES

I. P/CAF studies.

Cloning and characterization of P/CAF protein.

In human cells, CBP binds to c-Jun in a phosphorylation-dependent manner in association with stimulation of transcription (9). In yeast, GCN4 is believed to be a c-Jun counterpart on the basis of similarities in DNA recognition (15) as well as the participation of both proteins in UV signaling pathways (16). Yeast genetic screening has led to the isolation of various cofactors for GCN4, including GCN5 (yGCN5), ADA2 (yADA2) and ADA3 (yADA3) (17-19). These factors are considered to function as a complex (or in a common pathway) based on genetic and protein-protein interaction studies (18-22). Finally, p300/CBP and yADA2 exhibit significant sequence similarity within a 50 amino acid region including a Zn^{2+} finger motif (3). Human counterparts to yGCN5, yADA2, or yADA3 that interact with p300/CBP to mediate transcriptional activation by c-Jun were searched for in various nucleotide sequence databases.

Comparison of the yGCN5 protein sequence with various databases (23) revealed significant similarities with the two randomly sequenced human cDNAs, ETS05039 (24) ($P=4.0x10^{-15}$) and NIB2000-5R ($P=6.5x10^{-9}$). Given that these cDNAs were truncated, human fetal liver and fetal brain cDNA libraries (Clontech) were screened with ETS05039 and NIB2000-5R, respectively and complete clones were isolated from the human fetal liver cDNA library. The complete sequences revealed that the ETS05039- and NIB2000-5R-derived clones are encoded by distinct genes but are highly related within the protein coding regions (68% identity at the DNA level; 75% identity and 86% similarity at the protein level). The former encodes an N-terminal region with no sequence similarity to any proteins in the databases besides the yGCN5-related C-terminal region, whereas the latter encodes only the yGCN5-related region. Given that p300/CBP-binding activity was observed in the former polypeptide as shown below, it was designated p300/CBP-associated factor (P/CAF), having the amino acid

sequence of SEQ ID NO:1 and the nucleotide sequence of SEQ ID NO:10 and the latter was named human GCN5 (hGCN5), having the amino acid sequence of SEQ ID NO:5 and the nucleotide sequence of SEQ ID NO:11.

Additionally, an RNA blot (Clontech) was hybridized with a random-primed probe made from the cDNA encoding P/CAF. RNA blotting indicated that transcripts detected by the P/CAF and hGCN5 cDNAs are ubiquitously expressed, but the former is most abundant in heart and skeletal muscle, whereas the latter is most abundant in pancreas and skeletal muscle.

P/CAF-p300/CBP interaction *in vitro*

The P/CAF binding site was presumed to reside in the C terminal one third of CBP (residues 1,678-2,442) because it was observed that this region, when fused to a DNA binding domain, activates transcription (4) in a manner repressed by coexpression of 12S E1A. This region was divided into 6 overlapping fragments and each was expressed in *E. coli* as a glutathione-S-transferase (GST) fusion protein. GST-CBP fusions were incubated with recombinant P/CAF protein and, subsequently, purified using glutathione-Sepharose. Co-purified P/CAF was detected by immunoblotting analysis.

To construct GST-fusions, various regions of CBP and p300 were amplified by PCR. A series of deletions of the CBP segment B was created by site-directed *in vitro* mutagenesis (30). These fragments were subcloned into pGEX-2T (Pharmacia). GST-fusions were expressed in *E. coli* and extracted with buffer B [20 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 10% glycerol, 1 mM AEBSF, 0.1% NP40, 10 µg/ml of aprotinin, 10 µg/ml of leupeptin, 1 µg/ml of pepstatin A, 1 mM DTT] containing 0.1 M KCl for these experiments. GST-CBP-segment B was purified by glutathione-Sepharose and phenyl-Sepharose chromatographic steps, P/CAF, hGCN5, and E1A were expressed as FLAG-fusions in Sf9 cells via baculovirus vectors and affinity-purified with M2-agarose (ref. 30; Kodak-IBI). For interaction, a crude *E. coli* extract containing 20 pmol of GST-fusion was incubated with 40-60 pmol of P/CAF or E1A in a total volume of 50 µl of

buffer B with 0.1 M KCl on ice for 10 min. Samples were further incubated with 10 µl (packed volume) of glutathione-Sepharose at 4°C for 30 min, washed four times with 200 µl of buffer B containing 0.1 M KCl, and eluted with 20 µl of buffer E [50 mM Tris-HCl (pH 8.0), 0.2 M KCl, 20 mM glutathione] for 60 min. Interacting proteins were detected by anti-FLAG immunoblotting or silver staining.

For p300 interactions, the segment spanning residues 1763-1966 (segment B') of p300, which is analogous to the CBP segment-B, was used. Twenty percent of the P/CAF and hGCN5 inputs and 100% of the E1A input were also analyzed. In the GST precipitation assays, almost identical amounts of the GST fusions were recovered in all samples. Interaction between P/CAF and CBP (segment B) was determined in the absence and in the presence of E1A. Control reactions with GST-CBP alone and without GST-CBP were also performed. Input proteins were analyzed.

Two CBP segments, A and B, interacted specifically with P/CAF. The stronger interaction was observed in the latter segment, which does not include the yADA2-like Zn^{2+} finger. Given that the CBP segment-B is well conserved in p300 (66% identity, 75% similarity), the binding of P/CAF to p300 *in vitro* was also analyzed. For this experiment, the p300 segment spanning residues 1763-1966, termed segment B', which is analogous to the CBP segment-B, was used. Like CBP, p300 interacted specifically with P/CAF. These studies demonstrated that P/CAF binds specifically to both p300 and CBP *in vitro*. In contrast to P/CAF, hGCN5 did not bind to CBP or p300.

These studies also demonstrated that the Zn^{2+} finger region of p300/CBP, which shares sequence similarity with yADA2, is not essential for the interaction with P/CAF. Cloning of a human structural homolog of yADA2, termed hADA2 (25) has revealed that, unlike the sequence similarity between p300/CBP and yADA2, which is restricted to a 50 amino acid region, hADA2 shares extensive similarity (30% identity, 52% similarity) to yADA2 over the entire protein sequence. Moreover, a computer search of the complete genomic sequence of *Saccharomyces cerevisiae* revealed that yeast does

not have counterparts of p300/CBP or P/CAF. Thus, the p300/CBP-P/CAF pathway may have been acquired during metazoan evolution.

Action of E1A *in vitro*

Previous reports indicated that E1A binds to both the p300 segment spanning residues 1767-1816 and the CBP segment spanning residues 1805-1854 (7). These interactions were reconfirmed in the present system; thus, both p300 and CBP segments covering the previously identified regions interacted with E1A.

For further mapping, a series of deletions was introduced within the CBP segment-B and tested for interactions with P/CAF and E1A. Deletions of residues 1801-1825 or 1824-1851 markedly reduced interactions with both P/CAF and E1A, whereas deletion of residues 1850-1878 did not affect these interactions. Furthermore, deletion of residues 1801-1851 completely abolished interactions with both P/CAF and E1A. These data indicate that residues 1801-1851 of CBP are critical for interaction with both P/CAF and E1A. Taken together with the evidence that CBP segment A (aa residues 1,678-1,880) also binds to these factors, the above findings demonstrate that P/CAF and E1A bind to the same or very closely spaced sites on CBP.

Evidence that both P/CAF and E1A recognize the same p300/CBP segments raises the possibility of direct competition between P/CAF and E1A for binding to p300/CBP. To test this possibility, a competition experiment was performed with the use of affinity purified recombinant proteins. The interaction of P/CAF with the CBP-segment B was progressively inhibited by the addition of increasing amounts of E1A. In contrast, no inhibition was caused by an E1A mutant which does not bind to p300/CBP (E1AΔN). Similar results were obtained with the p300-segment B', leading to the conclusion that P/CAF and E1A compete for the same binding sites in p300/CBP.

P/CAF-p300/CBP interaction *in vivo*

The *in vivo* interaction between P/CAF and p300/CBP was established by co-immunoprecipitation from a human osteosarcoma cell extract. Proteins in this extract were immunoprecipitated with rabbit anti-P/CAF, rabbit anti-CBP and anti-p300 antibodies. For controls, cell extract was precipitated with rabbit control IgG or mouse anti-HA monoclonal antibody. The precipitates were analyzed by immunoblotting with anti-P/CAF, anti-CBP and anti-p300 antibodies.

Osteosarcoma cells were transfected with either control vector or E1A- or E1AΔN-expression vectors. Extract from the transfected subpopulation was immunoprecipitated with anti-P/CAF or control IgG. The precipitates were analyzed by immunoblotting with anti-p300 and anti-P/CAF antibodies.

Rabbit anti-P/CAF antibody was raised to the P/CAF segment spanning residues 125-397 and purified by immunoaffinity chromatography (33). A mixture of monoclonal antibodies raised to the human p300 segment spanning residues 1572-2371 (5) and rabbit polyclonal antibodies raised to the mouse CBP segment spanning residues 2-23 (for immunoprecipitation) and 1736-2179 (immunoblotting) were purchased from Upstate Biotechnology. Approximately 2 x 10^7 human osteosarcoma U-2 OS cells (ATCC accession number HTB 96) were extracted with 10 ml of lysis buffer [25 mM HEPES-KOH (pH 7.2), 150 mM potassium acetate, 2 mM EDTA, 1 mM DTT, 1 mM AEBSF, 10 μg/ml of aprotinin, 10 μg/ml of leupeptin, 1 μg/ml of pepstatin A, 20 mM sodium fluoride, 0.1% NP40]. Two to 10 ml of extract were incubated with 2 μg of the respective antibody for four hours at 4°C. Fifty μl (packed volume) of protein-A Trisacryl (Pierce) were added and incubation was continued for two hours. The matrix was washed four times with 1 ml of the lysis buffer, then boiled in 2x SDS sample buffer. Human osteosarcoma U-2 OS cells were transfected with 20 μg of the indicated plasmid and 1 μg of sorting plasmid (pCMV-IL2R) (31). The transfected subpopulation was purified by magnetic affinity cell sorting (32). Extract from approximately 2 x 10^5 sorted cells was immunoprecipitated as described.

Anti-P/CAF antibody specifically detected a 95 kDa protein, which is very close to the calculated value for the full-length P/CAF, in the immunoprecipitates. Anti-P/CAF antibody co-immunoprecipitated both CBP and p300. Similarly, anti-CBP antibody also co-immunoprecipitated P/CAF. However, anti-p300 antibody did not co-immunoprecipitate P/CAF. This is most likely due to steric interference since the anti-p300 antibody was raised to the p300 segment spanning residues 1572-2371 which includes the P/CAF binding region. These data demonstrate that P/CAF forms complexes with both p300 and CBP *in vivo*.

Action of E1A *in vivo*

The *in vitro* experiments described herein indicate that P/CAF and E1A compete for the binding sites in p300/CBP. Thus, a study was conducted to determine whether E1A targets the endogenous interaction between P/CAF and p300. An E1A-expression vector was transiently transfected into human osteosarcoma cells and the transfected subpopulation was purified by cell sorting. Then, the interaction between P/CAF and p300 in transfected cells was examined by co-immunoprecipitation with anti-P/CAF antibody. The endogenous interaction of P/CAF with p300 was drastically inhibited by expression of E1A. On the other hand, no inhibition was observed by the E1A mutant lacking the p300 binding domain (E1AΔN), indicating that E1A disrupts the P/CAF-p300 complex *in vivo* through an interaction with p300.

Cell cycle regulation by P/CAF

Given that binding of P/CAF to p300/CBP is inhibited by E1A, experiments were performed to evaluate whether P/CAF, by binding to and forming a functional complex with p300, is involved in the regulation of entry into S phase. This possibility was addressed by examining whether transient expression of P/CAF would affect the rate of G1/S transit in HeLa cells. P/CAF negatively affected the distribution of cells between G1 and S phases in this assay.

HeLa cells were transfected by electroporation with 7 μg of P/CAF-expression plasmid and/or 3 μg of the full-length or the N-terminally deleted (Δ2-36) E1A 12S-

expression plasmid as indicated. These plasmids were constructed by subcloning FLAG-P/CAF and E1A cDNAs into pCX (34) and pcDNAI (Invitrogen), respectively. All samples, in addition, contained 1 μg of sorting plasmid (pCMV-IL2R) (31) and carrier plasmid (pCX) to normalize the total amount of DNA to 11 μg. After transfection, cells were incubated in Dulbecco's modified Eagle's medium with 10% fetal bovine calf serum for 12 h, and subsequently labeled in medium containing 10 μM bromo-deoxyuridine (BrdU) for 30 min. Subsequently, the transfected subpopulation was purified by magnetic affinity cell sorting and nuclei were analyzed by dual parameter flow cytometry as described (32).

The fraction of cells accumulating in S phase in control cultures was 23%, compared to 15% in P/CAF-transfected cells. This effect was reproducible in multiple independent experiments. In parallel experiments to verify the utility of this experimental protocol, plasmids encoding E2F-1, simian virus 40 small t, cyclin A or cyclin E increased the accumulation of cells in S phase, whereas plasmids encoding the cyclin-dependent kinase inhibitors p21 or p27 reduced the number of S phase cells.

On the basis of evidence that E1A and P/CAF compete for binding sites on p300, it seemed possible that cotransfection of P/CAF with E1A would oppose the mitogenic effect caused by E1A. As shown by the data herein, this is indeed the case. E1A alone has mitogenic activity in this experimental setting, while the E1A mutant lacking the p300 binding domain (E1AΔN) has very weak activity. Comparable expression levels between wild type and mutant E1A in the transfected cells were revealed by immunoblotting analysis with anti-E1A. Intriguingly, when P/CAF was cotransfected with E1A, the mitogenic activity of E1A was significantly counteracted by P/CAF. These results show that P/CAF and E1A mediate antagonistic effects on cell cycle progression.

In the course of assessing P/CAF activity, it was also revealed that p300 is able to inhibit cell cycle progression under the same assay conditions. These findings suggest

that P/CAF and p300, perhaps by forming a complex, act in concert to suppress cell cycle progression.

Histone acetyltransferase activity in P/CAF

Acetylation of the N-terminal histone tails has been considered to play a crucial role in accessibility of transcription factors to nucleosomal templates (26-27). Recently, yGCN5 has been identified as a histone acetyltransferase (28). On the basis of this information, intrinsic histone acetyltransferase activity in P/CAF and hGCN5 was examined. As substrates, the core histones (histones H2A, H2B, H3 and H4) and the nucleosome core particles (146 base pairs of DNA wrapped around the octamer of core histones) were used.

Activity of hGCN5 and P/CAF that acetylates free histones or histones in the nucleosome core particle (35) was measured as described (36). Each reaction contained 0.3 pmol of affinity purified FLAG-hGCN5 or FLAG-P/CAF, 4 pmol of the histone octamer or the nucleosome core particle and 10 pmol of [1-^{14}C]acetyl-CoA. The histone octamer dissociated into dimers or tetramers under assay conditions. Acetylated histones were detected by autoradiography after separation by SDS-PAGE.

P/CAF and hGCN5 acetylated the core histones with almost the same efficiency. Both factors acetylated histones H3 and H4, but preferentially H3. In contrast, very weak or no acetylation by hGCN5 was detected in the nucleosome core particles. Remarkably, significant acetylation by P/CAF was observed in a nucleosomal context. Although all core histones are acetylated in the nucleus, P/CAF and hGCN5 did not acetylate histones H2A and H2B *in vitro*.

Direct function of P/CAF is likely to involve its intrinsic histone acetyltransferase activity. Although exact molecular mechanisms by which acetylation of core histones contribute to transcription remains undefined, acetylation of the histones is considered to play an important role in transcriptional regulation (26-27). The positively charged N-terminal tails of core histones are believed to affect nucleosome structure by interacting

with DNA at or near the nucleosome-spacer junction. Acetylation of the histone tails presumably destabilizes the nucleosome and facilitates access by regulatory factors. Likewise, there is a general correlation between the level of acetylation and transcriptional activity of nucleosomal domains. The findings of the present invention provide insights into the mechanisms of targeted histone acetylation.

Cellular factor p300/CBP binds to various sequence-specific factors that are involved in cell growth and/or differentiation, including CREB (3,4), c-Jun (9), Fos (11), c-Myb (12) and nuclear receptors (13). P/CAF could stimulate the activation function of these factors via promoter-specific histone acetylation. The present invention demonstrates that E1A appears to perturb normal cellular regulation by disrupting the connection between p300/CBP and its associated histone acetyltransferase.

II. p300/CBP studies.

Purification of E1A associated histone acetyltransferase.

FLAG-epitope tagged E1A (or ΔE1A) was expressed in Sf9 cells (ATCC accession number CRL 1711) by infecting recombinant baculovirus (43). All purification steps were carried out at 4°C. Extract was prepared from infected cells by one cycle of freeze and thaw in buffer B (20 mM Tris-HCl, pH 8.0; 5 mM $MgCl_2$; 10% glycerol; 1 mM PMSF; 10 mMβ-mercaptoethanol; 0.1% Tween 20) containing 0.1 M KCl and the complete protease inhibitor cocktail (Boehringer Mannheim). To prepare E1A-immobilized beads, the extract was incubated with M2 anti-FLAG antibody agarose (Kodak-IBI) for four hours with rotating and subsequently washed with the same buffer three times. The resulting beads were incubated with HeLa (ATCC accession number CCL 2) nuclear extract for four to eight hours and thereafter washed with the same buffer six times. Finally, FLAG-E1A was eluted from the beads along with associated polypeptides by incubating with the same buffer containing 0.1 mg/ml FLAG peptide.

For further purification, eluted polypeptides were dialyzed in 0.05 M KCl-buffer B and subsequently loaded onto a SMART Mono Q column (Pharmacia) equilibrated with the same 0.05 M KCl-buffer B. After washing, the column was developed with a linear gradient of 0.05-1.0 M KCl in buffer B. Mono Q fractions were concentrated with a MICROCON spin-filter (Amicon) and consequently loaded onto a SMART Superdex 200 column (Pharmacia) equilibrated with 0.1 M KCl-buffer B.

Histone acetyltransferase assays

Filter binding assays were performed as described (80) with minor modifications. Samples were incubated at 30°C for 10-60 minutes in 30 ml of assay buffer containing 50 mM Tris-HCl, pH 8.0; 10% glycerol; 1 mM DTT; 1 mM PMSF; 10 mM sodium butyrate; 6 pmol of [^{3}H]acetyl CoA (4.3 mCi/mmole, Amersham Life Science Inc.); and 33 mg/ml of calf thymus histones (Sigma Chemical Co.). In experiments where synthetic peptides were substituted for core histones, 50 pmol of each peptide were used. After incubation, the reaction mixture was spotted onto Whatman P-81 phosphocellulose filter paper and washed for 30 minutes with 0.2 M sodium carbonate buffer pH 9.2 at room temperature with 2-3 changes of the buffer. The dried filters were counted in a liquid scintillation counter.

PAGE analysis was done as above except that 90 pmol of [^{14}C]acetyl CoA (55 mCi/mmole, Amersham Life Science Inc.) and 9 pmol of core histones or mononucleosomes were used. Core histones and mononucleosomes were prepared as described (35). For trypsin digestion, reaction mixtures were further incubated with various amounts of trypsin on ice for 30 minutes. The samples were analyzed on one dimensional SDS-PAGE gels or two dimensional gels, where the first dimension was an acid-urea-PAGE gel (44) and the second dimension was an SDS-PAGE gel.

Protein expression

For baculovirus expression, cDNAs corresponding to p300 portions of aa 1-670, aa 671-1194 and aa 1135-2414 were amplified by PCR (EXPAND High Fidelity PCR System; Boehringer Mannheim) as KpnI-NotI fragments. The resulting fragments were

subcloned into a baculovirus transfer vector having the FLAG-tag sequence (43). The recombinant viruses were isolated using the BACULOGOLD system (Pharmingen), according to the manufacturer's protocol and were infected into Sf9 cells (ATCC accession number CRL 1711) to express FLAG-p300. Recombinant proteins were affinity purified with M2 anti-FLAG antibody-immobilized agarose (Kodak-IBI) according to the manufacturer's protocol.

For bacterial expression, cDNAs encoding the p300 portions and the CBP portion (aa 1174-1850) were first subcloned into the baculovirus transfer vector having the FLAG-tag as described above. Thereafter, the XhoI and NotI fragments encoding FLAG-p300 or FLAG-CBP fusions were resubcloned into the *E. coli* expression vector pET-28c (Novagene) digested with SalI and NotI. Recombinant proteins were expressed in *E. coli* BL21(DE3) and affinity purified with M2-antibody agarose.

Histone acetyltransferases that associate with E1A

Although the adenovirus E1A 12S protein (E1A) inhibits transcription in a variety of genes via direct binding to p300/CBP (45), E1A also stimulates transcription in some contexts (46). Thus, p300/CBP-bound E1A was tested to determine whether it might recruit histone acetyltransferases or deacetylases to regulate transcription. In addition, experiments were conducted as described below to determine if p300/CBP per se is a histone acetyltransferase.

Initially, recombinant FLAG-epitope tagged E1A was immobilized on anti-FLAG antibody beads. Immobilized E1A was incubated with a HeLa nuclear extract for affinity purification of E1A-associated polypeptides. FLAG-E1A was then eluted from the beads, along with E1A-associated polypeptides, by incubating with FLAG-peptide. Although E1A per se has no histone acetyltransferase activity, E1A recruited significant amounts of histone acetyltransferase activity from the nuclear extract. It is very unlikely that this activity is derived from P/CAF given that E1A and P/CAF cannot bind to p300/CBP simultaneously (43). Consistent with this, no P/CAF was detected in these fractions by immunoblotting.

The E1A N-terminus, a region that is not highly conserved among the various adenovirus serotypes, is involved in p300/CBP binding *in vivo*. Mutations in the N-terminal region lead to loss of the ability for p300/CBP binding without affecting RB binding (1,47). Thus, the requirement of the E1A N-terminal region for the recruitment of histone acetyltransferase activity was tested. In contrast to the wild type, the N-terminal deleted form of E1A (ΔN-E1A) recruited only a background level of acetyltransferase activity. In agreement with previous reports (47), the ΔN-E1A showed no ability to interact with p300/CBP, although it still retained the ability to interact with a variety of other polypeptides, including RB.

To define the relationship between p300/CBP and histone acetyltransferase activity, affinity purified E1A-binding polypeptides were separated by Mono Q ion-exchange column. Both p300/CBP and the acetyltransferase activity were coeluted at 140 mM KCl, while most of polypeptides were eluted at 260 mM KCl. The active fraction of Mono Q column (~140 mM KCl) was further separated by Superdex-200 gel filtration column. Both p300/CBP and the acetyltransferase activity coeluted after the void volume, indicating that p300/CBP is involved in the histone acetyltransferase activity.

p300 is a histone acetyltransferase

The data provided herein indicate that p300 per se, or a polypeptide(s) associated with p300, possesses histone acetyltransferase activity. To test the former possibility, the acetyltransferase activity of recombinant p300 was measured. p300 was divided into three fragments, each of which was expressed in and purified from Sf9 cells via a baculovirus expression vector. Histone acetyltransferase activity was readily detected in the C-terminal fragment containing amino acids 1135-2414, whereas no activity was found in the other fragments, demonstrating conclusively that p300 per se is a histone acetyltransferase.

p300/CBP-histone acetyltransferase domain

To map the histone acetyltransferase domain of p300, a series of deletions was prepared. Given the poor conservation of the glutamine-rich region (aa 1815-2414) in the *C. elegans* p300/CBP homolog (6), the p300 fragment encoding aa 1135-1810 was expressed in and purified from *E. coli*. Importantly, this candidate region of p300 (aa 1135-1810) showed significant histone acetyltransferase activity. For further mapping within this region, a series of N-terminal deletions was constructed. Deletion of 60 residues, resulting in a fragment containing aa 1195-1810, had no effect on the acetyltransferase activity, whereas the deletion of 185 residues, yielding a fragment comprising aa residues 1320-1810, completely eliminated the acetyltransferase activity.

Next, a series of C-terminal deletions was analyzed to determine the requirement of the P/CAF (or E1A) -binding domain. The p300 fragments lacking the E1A binding domain (aa 1195-1760, 1195-1706 and 1195-1673) still retained the acetyltransferase activity, whereas the further truncated mutant (aa 1195-1652) completely lost the acetyltransferase activity. Consistent with these results, the internal deletion of residues 1418-1720 showed no acetyltransferase activity. These data demonstrate that the histone acetyltransferase domain is located between the bromodomain and the E1A-binding domain. Given that the histone acetyltransferase domain is highly conserved between p300 and CBP (91% similarity), the corresponding region of CBP, aa residues 1174-1850, was expressed to confirm the acetyltransferase activity. As expected, comparable activity was detected, indicating that both p300 and CBP are histone acetyltransferases.

Among various acetyltransferases including histone acetyltransferases GCN5 and P/CAF, putative acetyl-CoA binding sites are conserved (48). However, multiple alignment analysis (49) showed that the p300/CBP histone acetyltransferase domain does not belong to this group. Moreover, comparison of the p300/CBP histone acetyltransferase domain with peptide sequence databases (23) showed no sequence similarity to any other proteins. Accordingly, this invention shows that p300/CBP represents a novel class of acetyltransferases in that it does not have the conserved motif found among previously described acetyltransferases (48).

p300 acetylates all core histones in mononucleosomes

Substrate specificity for acetylation by p300 was also examined. As substrates, histone octamers and mononucleosomes (146 base pairs of DNA wrapped around the octamer of core histones) were used. Given that the histone octamer dissociates into dimers or tetramers under physiological conditions, the histone octamer is referred to here as core histones. When core histones were used, p300 acetylated all four proteins, but preferentially H3 and H4. More importantly, in a nucleosomal context, p300 acetylated all four core histones nearly stoichiometrically. In contrast, p300 acetylated neither BSA nor lysozyme.

Hyperacetylated histones are believed to be linked with transcriptionally active chromatin (26,27,50,51). Hyperacetylated forms are found in histones H4, H3 and H2B, which have multiple acetylation sites *in vivo*. Thus, the level of acetylation by p300 was also tested.

Mononucleosomes treated with p300 were analyzed by two-dimensional gel electrophoresis. A Coomassie blue-stained gel and the corresponding autoradiogram showed that a significant amount of histones, especially H4, were hyperacetylated. Importantly, acetylation levels by p300 were very close to those of hyperacetylated histones prepared from HeLa nuclei treated with sodium butyrate, a histone deacetylase inhibitor. In contrast, no acetylated forms were detected in the reaction without p300. These results indicate that p300 acetylates histones in mononucleosomes to the hyperacetylated state by targeting multiple lysine residues.

p300 acetylates the four lysines in the histone H4 N-terminal tail *in vitro* which are acetylated *in vivo*

Lysines at positions 5, 8, 12 and 16 of histone H4 are acetylated *in vivo* (51). Recent studies with yeast histone acetyltransferases demonstrate the position-specific acetylation by distinct acetyltransferases, i.e., while cytoplasmic acetyltransferases for histone deposition and chromatin assembly modify

positions 5 and 12, GCN5 modifies positions 8 and 16 (52). Accordingly, the positions of acetylation by p300 were also determined. A series of synthetic peptides containing acetylated lysines at various positions was used to determine the acetylation site-specificity of p300. Consistent with the two-dimensional gel electrophoresis analysis, the experiments with peptide substrates showed that p300 acetylates all four lysines in the histone H4 that are acetylated *in vivo*. These results are consistent with the view that deposition-related diacetylated histones are deacetylated during maturation of chromatin (53).

p300 preferentially acetylates the N-terminal histone tail

Histone acetyltransferases modify specific lysine residues in the N-terminal tail of core histones but not the C-terminal globular domain *in vivo* (26,27,50,51). Structural models of nucleosomes (54,55,56) suggest that most of the lysine residues in the C-terminal globular domain are buried. Therefore, experiments were conducted to examine whether restricted acetylation of the N-terminal tail resulted from the substrate specificity of the enzyme or inaccessibility of the enzyme to the core domain in nucleosomes. The globular domains of all core histones contain a long helix flanked on either side by a loop segment and short helix, termed the "histone fold" (54,55,56). The histone fold is involved in formation of the stable H2A-H2B and H3-H4 hetero-dimers, consisting of extensive hydrophobic contacts between the paired molecules. Therefore, it is likely that a histone monomer cannot fold properly, thereby increasing access of the histone acetyltransferase to the core domain. Based on these considerations, experiments were conducted to determine whether p300 acetylates free histone H4 in a N-terminal-specific manner.

Histone H4 was acetylated with p300 and subsequently the histone tail was removed by partial digestion with trypsin. The distributions of radioactivity between intact and core histones were compared. While the globular core histone domain was predominant at the higher trypsin concentrations, radioactivity was detected mostly in the intact histone. These data demonstrate that p300 preferentially acetylates the N-terminal tail of histone H4.

III. P/CAF interaction with MyoD

Tissue culture and transfection experiments

C_2C_{12} mouse cells (ATCC accession number CRL 1772) were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum (FBS) until they reached confluence. Differentiation was induced by switching medium to differentiation medium (DM), consisting of DMEM containing 2% horse serum. $C_3H/10T1/2$ fibroblasts (ATCC accession number CCL 226) were grown in DMEM supplemented with 10% FBS. Cells were transfected by the calcium phosphate precipitation method. Total amounts of transfected DNA were equalized by empty vector DNA. After 12 h incubation in medium containing the precipitated DNA, the cells were washed and incubated in fresh DMEM containing 10% FBS for an additional 24 h. Afterwards, differentiation was induced by incubating in DM for 36 to 72 h. Chloramphenicol acetyltransferase (CAT) assays were performed as previously described (64,69). The quantities of cell extracts used for CAT assays were normalized to β-galactosidase activity by cotransfection of 1 mg of the β-galactosidase expression vector, pON260.

Expression vectors used for transfection experiments are as follows: pCX-P/CAF for P/CAF (43); pCMV-bp300 for p300 (65), pCMV-p300 (1869-2414) (64) and pCMV-p300 (1514-1922) (60) for p300 wild type and mutants; pE1A12S, pE1A12S R2G, pE1A12S D2-36 and pE1A12S D121-130 for E1A wild type and mutants (66,67,68); and pEMSV-MyoD for MyoD (64).

The antisense P/CAF RNA expression vector, pcDNA3 P/CAF-AS, was created as follows. The 2.5 Kb EcoRI-KpnI fragment containing the entire P/CAF open reading frame was isolated from pCX-P/CAF (43). This fragment was subcloned into the

EcoRI-KpnI sites of plasmid pcDNA3 (Invitrogen) so that the antisense P/CAF RNA is driven under the CMV promoter. Reporter genes employed were 4RE-CAT and MCK-CAT (69). 4RE-CAT is driven by a synthetic promoter containing 4 copies of the E-box, whereas MCK-CAT is driven by the native MCK promoter (nucleotides -1256 to +7).

Microinjection and immunofluorescence

Cells were grown on small glass slides, subdivided into numbered squares of 2 mm x 2 mm and microinjected with purified and concentrated antibodies, as previously described (70). For immunofluorescence, cells were fixed in either 2% paraformaldehyde or 1:2 methanol/acetone solution, preincubated with 5% BSA/PBS and incubated with the primary antibodies for 30 min at 37° C. Subsequently, antibody was visualized by incubating with either rhodamine- or fluorescein-conjugated secondary antibody for 30 min at 37° C. Injected antibodies were stained with a rhodamine-conjugated secondary antibody and nuclei were counter-stained by DAPI as previously described (69).

Antibodies employed are as follows; rabbit polyclonal affinity purified anti-P/CAF antibody (43), rabbit polyclonal anti-p300/CBP antiserum (71), mouse monoclonal anti-MyoD antibody (clone 5.8A, kindly provided by P. Houghton), goat polyclonal anti-c-Jun affinity purified antibody (Santa Cruz) and rabbit pre-immune serum.

Immunoprecipitation and DNA affinity purification

Cells were resuspended in lysis buffer (20 mM $NaPO_4$, 150 mM NaCl, 5mM $MgCl_2$, 0.1% NP40, 1 mM DTT, 10 mM sodium fluoride, 0.1 mM sodium vanadate, 1 mM phenylmethylsulfonyl-fluoride and 10 mg/ml each of leupeptin, aprotinin and pepstatin). After 30 min incubation on ice, samples were centrifuged at 12,000 x g for 30 min and supernatants were used as cell extracts. Extracts were pre-cleared by

incubating with rabbit pre-immune serum and protein A/G Plus-Agarose (Santa Cruz) for 2 h at 4 C. For immunoprecipitation, the supernatants were incubated with the respective antibodies for 3 h at 4 C. Protein A/G Plus-Agarose was added, and incubation continued for 3 h. The matrix was washed with lysis buffer, then boiled in 2 X SDS sample buffer. Immunoblotting was performed by using the ECL chemiluminescent detection kit (Amersham) according to the manufacturer's protocol.

Affinity purification of E-box-bound complexes was done as previously described (69). Briefly, 100 ng of the biotinylated double stranded DNA containing the E-box were immobilized on streptavidin-conjugated magnetic beads and incubated with 500 mg of cell extracts in the presence of poly dI-dC. After extensive washing, bound proteins were eluted with SDS sample buffer and analyzed by immunoblotting.

In vitro protein-protein interaction assays

The CBP-B fragment and its deletion derivatives were expressed as GST-fusions described previously (43). MyoD and E1A (43) were expressed as FLAG-fusion proteins in Sf9 cells via a baculovirus expression system and affinity-purified on M2 anti-FLAG antibody-agarose (Kodak-IBI). Crude E. coli extracts containing GST-fusions were incubated with various amounts of MyoD and/or E1A in 50 ml of buffer B (20 mM Tris-HCl, pH 8.0, 0.1 M KCl, 5 mM $MgCl_2$, 10% glycerol, and 0.1% Nonidet P-40) on ice for 10 min. GST-precipitation was performed as described (43). MyoD and E1A were detected by immunoblotting with anti-FLAG M2 antibody. For the interaction between P/CAF and MyoD, 1.5 pmol of FLAG-P/CAF and 15 pmol of FLAG-MyoD were incubated in 50 ml of buffer B on ice for 10 min. The mixture was further incubated with 2 mg of anti-P/CAF (43) or anti-hADA2 antibody for 60 min. The immunocomplexes were precipitated by incubation with [illegible] ml of protein A-Trisacryl (Pierce) and rotated for 1-4 hr at 4oC. The matrix was washed 4 times with 200 ml of buffer B and boiled in 10 ml of 2 X SDS sample buffer. The proteins were resolved on a 4%-20% gradient SDS-PAGE and subjected to immunoblotting with the anti-FLAG M2 antibody. The blot was developed with the SUPERSIGNAL chemiluminescent substrates (Pierce).

P/CAF coactivates muscle-specific transcription

P/CAF and MyoD were co-transfected into mouse C3H10T1/2 fibroblasts, and MyoD-mediated transcription was determined from reporter activity driven by the artificial (4RE) and the naturally-occurring muscle creatine kinase (MCK) promoters. Overexpression of P/CAF stimulated MyoD-dependent transcription several folds in both promoters. Similar results were obtained for the myoD activated myogenin promoter Transcriptional activation was further stimulated by co-transfecting with MyoD, P/CAF and p300 expression vectors, suggesting that P/CAF may function by forming a complex with p300/CBP. Consistent with the lack of DNA binding capacity in P/CAF, overexpression of P/CAF alone did not increase the basal transcriptional activity of either enhancer. To test whether P/CAF and p300/CBP function in the same pathway, two dominant negative forms of p300 were employed which specifically inhibit p300/CBP-mediated transcription (60,64). The p300 segment spanning residues 1514-1922 inhibits the MyoD-dependent activation via direct interaction with MyoD (60), whereas the p300 segment spanning residues 1869-2414 inhibit it without direct interaction (64). Both dominant negative mutants inhibited MyoD-coactivation by P/CAF), suggesting that P/CAF and p300/CBP function in the same pathway.

For further elucidation of the activation mechanism by P/CAF, the effect of E1A, which inhibits MyoD-dependent transcription and differentiation (66,72,73) via direct interaction with p300/CBP (65,78), was tested. Expression of E1A in C3H10T1/2 fibroblasts inhibited stimulation of MyoD-directed transcription by P/CAF overexpression. E1A mutants lacking p300/CBP-binding activity, E1A D2-36 and E1A R2G (67,79), had almost no effect. On the other hand, an E1A mutant retaining p300/CBP-binding activity, E1A D121-130, behaved like the wild type. Since E1A associates with p300/CBP, but not with P/CAF, these results suggest that P/CAF functions in MyoD-directed transcription via interaction with p300/CBP.

To address the role of P/CAF as a myogenic coactivator in a more relevant environment, P/CAF was overexpressed in proliferating C2C12 myoblasts which express

endogenous myogenic bHLH factors. As observed in fibroblasts, overexpression of P/CAF stimulated muscle specific transcription. Concomitant expression of exogenous p300 increased P/CAF-mediated coactivation. The repression exerted by wild type E1A, but not mutant E1A D2-36, on P/CAF coactivation of MyoD was also observed in muscle cells.

Similar experiments were performed with myogenic cell lines that were stably transformed with wild type or mutant E1A-expressing vectors (66). Coactivation by P/CAF was inhibited by wild type E1A or the E1A mutant that retains p300/CBP-binding activity (E1AΔ121-130). In contrast, E1A mutants that lack p300/CBP-binding (E1A Δ2-36 and E1A R2G) allowed transcriptional coactivation by P/CAF. Taken together, these experiments show that P/CAF coactivates MyoD-directed transcription via interaction with p300/CBP.

P/CAF stimulates myogenic differentiation

Given that P/CAF potentiates MyoD-directed transcription, the ability of P/CAF to assist MyoD in promoting myogenic differentiation was investigated. To this aim, C3H10T1/2 fibroblasts were transiently transfected with P/CAF and MyoD expression vectors. An expression vector for the green fluorescent protein (GFP) was co-transfected to identify transfected cells. After incubation in differentiation medium, the myogenic conversion of transfected cells was determined by simultaneous expression of the GFP and the differentiation-specific marker myosin heavy chain (MHC). Forced expression of MyoD in fibroblasts caused muscle differentiation in 12% of the transfected fibroblasts. This myogenic conversion was 20% by co-expressing MyoD and P/CAF. As observed in transcription experiments, stimulation of differentiation by P/CAF was counteracted by co-transfection with the p300 dominant negative mutant, p300 (1869-2414). Consistent with a general role for coactivators, overexpression of P/CAF alone was unable to differentiate fibroblasts.

Similar experiments were done using proliferating C2C12 myoblasts in which the differentiation program is already committed. Most of the myoblasts differentiated into

myotubes by overexpressing P/CAF, whereas only a modest effect was observed by overexpressing p300. In contrast, differentiation was inhibited slightly by overexpressing c-Jun. This inhibitory effect presumably was caused by titration of p300/CBP, which associates directly with c-Jun (74). A similar inhibition was observed in the p300 dominant negative mutant. Consistent with the transcriptional effect, E1A almost completely inhibited differentiation. The E1A mutant RG2, lacking p300/CBP-binding capability but retaining the retinoblastoma protein (Rb)-binding capability, only partially inhibited differentiation, although this same mutant inhibited transcription as severely as the wild type. Taken together, these data show that P/CAF stimulates muscle differentiation by coactivating MyoD function via p300/CBP association.

P/CAF is essential for myogenic transcription and differentiation

To test the necessity of P/CAF for myogenic transcription, experiments were conducted whereby P/CAF synthesis was inhibited by expressing antisense P/CAF RNA. A vector from which the P/CAF mRNA is transcribed in the antisense orientation (P/CAF-AS) was transfected with P/CAF and MyoD expression vectors into fibroblasts and MyoD-dependent transcription was examined. Cotransfection of the antisense expression vector strongly inhibited MyoD-dependent transcription below the level of induction elucidated by MyoD alone, demonstrating that expression of P/CAF antisense RNA inhibits not only the coactivation exerted by exogenous P/CAF but also that of endogenous P/CAF. These results indicate that P/CAF is essential for MyoD-dependent transcription.

Studies were also carried out to determine whether expression of P/CAF antisense RNA inhibits myogenic differentiation. C3H10T1/2 fibroblasts were transiently transfected with various expression vectors with or without the P/CAF antisense RNA expression vector. Expression of P/CAF antisense RNA reduced MyoD-mediated myogenic conversion of fibroblasts. Expression of P/CAF antisense RNA also counteracted the stimulatory effect of both P/CAF and p300 on myogenic differentiation. These data support the view that P/CAF and p300/CBP coactivate

MyoD-dependent transcription in the same pathway. More drastic inhibition was observed in C2C12 myoblasts in similar experiments. Therefore, it can be concluded that P/CAF is essential for transcription of muscle specific genes and hence differentiation into myotubes.

To further confirm the essential role of P/CAF for myogenic differentiation, we blockage experiments by antibody microinjection were performed. Antibodies were injected into the cytoplasm of proliferating C2C12 myoblasts to prevent the nuclear transport of newly synthesized target proteins. After incubating in the differentiation medium, the degree of differentiation was determined. Microinjection of an anti-P/CAF antibody almost completely inhibited differentiation. Similar results were obtained by microinjecting anti-p300/CBP antibodies. Although microinjection of either anti-p300/CBP or P/CAF antibody was sufficient to inhibit differentiation, an even greater inhibition was observed by coinjecting both of them. Microinjection of anti-P/CAF or anti-p300/CBP antibody did not interfere with induction of p53 by DNA damaging agents, showing specificity of the inhibition by the antibodies. In contrast to anti-P/CAF or anti-p300/CBP antibodies, the injection of anti-MyoD antibody only partially inhibited differentiation, supporting the view of functional redundancy between MyoD and Myf-5 (75,76). Injection of anti-c-Jun antibody or control antibody did not interfere with muscle differentiation.

Similar experiments were performed with C3H10T1/2 fibroblasts stably expressing MyoD. In these cells, either anti-p300/CBP or anti-P/CAF antibody completely inhibited muscle differentiation. In contrast to myoblasts, anti-MyoD antibody completely blocked differentiation in the fibroblasts expressing MyoD. Anti-c-Jun and control antibodies did not interfere with differentiation. Taken together, these results demonstrate that P/CAF and p300/CBP are indispensable for activation of the myogenic program.

p300/CBP, P/CAF and MyoD form a multimeric complex *in vivo*

The data described above indicate that P/CAF stimulates MyoD-directed transcription via association with p300/CBP. Thus, experiments were conducted to investigate whether P/CAF, p300/CBP and MyoD could associate in a complex. First, cellular extracts derived from C2C12 myotubes were subjected to immunoprecipitation. Both anti-MyoD and anti-p300/CBP antibodies co-precipitated P/CAF. In a complementary experiment, both anti-p300/CBP and anti-P/CAF antibodies also co-precipitated MyoD, suggesting that these factors form a multimeric protein complex in myotubes.

Next, attempts were made to detect this complex on the E-box, the DNA binding site for MyoD. Immobilized DNA containing an E-box sequence was incubated with myotube extracts. After extensive washing, P/CAF, p300/CBP and MyoD were analyzed by immunoblotting. P/CAF, p300/CBP and MyoD were all affinity purified on the immobilized DNA, whereas they were not purified on the control DNA lacking the E-box. Given that P/CAF and p300/CBP per se cannot bind to DNA, these observations indicate that P/CAF and p300/CBP are recruited through MyoD at the E-box sites to form a multi-protein complex.

Complex formation is inhibited by viral transforming factors

Since the oncoviral proteins E1A and large T antigen inhibit myogenic transcription and differentiation, the effect of these factors on the formation of complexes on the E-box was tested. Importantly, very small amouts of P/CAF and p300/CBP were co-purified on the E-box from myocyte extracts which stably express E1A or large T antigen, although MyoD was detected under these conditions. The lower recovery of MyoD from E1A-expressing muscle cells could reflect the low level of MyoD in the extracts (66). These results indicate that E1A and large T antigen dissociate P/CAF and p300/CBP from MyoD without altering MyoD binding to DNA.

Consistent with the previous observations that transiently expressed E1A prevents interaction between P/CAF and p300/CBP *in vivo* (43), the association

between p300/CBP and P/CAF was abolished in myoblasts stably transformed by wild type E1A but not in those clones transformed with the E1A mutant R2G unable to bind p300/CBP. Similarly, the interaction between p300/CBP and P/CAF was abolished by large T antigen but not by the mutant protein that localizes into the cytoplasm (77).

Interaction between MyoD, P/CAF and CBP *in vitro*

Previous interaction experiments *in vitro* indicate that the CBP region spanning residues 1801 to 1850 is crucial for interaction with both P/CAF and E1A (43). While most sequence-specific factors bind to CBP sites distinct from the P/CAF/E1A binding sites, MyoD interacts with an overlapping CBP fragment called the CH3 region (60,64,65). To understand how P/CAF, p300/CBP and MyoD associate, the CBP sites important for MyoD binding were mapped more precisely. Consistent with previous reports (60,64,65), the CBP fragment spanning residues 1801-2000 (fragment B) bound MyoD. Moreover, deletion of residues 1801 to 1850 within fragment B completely abolished interaction with MyoD, which is similar to the results obtained with P/CAF and E1A. Importantly, an internal deletion of residues 1850-1878 abolished the MyoD interaction with CBP, while it did not affect binding of E1A or P/CAF (43). These results suggest that MyoD and P/CAF bind to distinct sites of p300/CBP, albeit the binding sites may overlap. Moreover, a direct interaction was observed between MyoD and P/CAF, which may contribute to stabilization of the multimeric complex.

These data show that E1A prevents not only p300/CBP-interaction with P/CAF but also that with MyoD *in vivo*. To obtain evidence that this inhibition is due to the direct action by E1A, competition experiments were performed *in vitro*. Importantly, the interaction between CBP and MyoD was strongly inhibited by addition of E1A, implicating that E1A inhibits myogenic transcription by disrupting multiple interactions.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be

regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Throughout this application various publications are referenced by numbers within parentheses. Full citations for these publications are as follows. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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SEQUENCE LISTING

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(v) COMPUTER READABLE FORM:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Pro Pro Ala Pro Pro Gln Gly Ser Pro Cys Ala Ala Ala Ala Gly Gly
        35                  40                  45
Ser Gly Ala Cys Gly Pro Ala Thr Ala Val Ala Ala Ala Gly Thr Ala
    50                  55                  60
Glu Gly Pro Gly Gly Gly Gly Ser Ala Arg Ile Ala Val Lys Lys Ala
65                  70                  75                  80
Gln Leu Arg Ser Ala Pro Arg Ala Lys Lys Leu Glu Lys Leu Gly Val
                85                  90                  95
Tyr Ser Ala Cys Lys Ala Glu Glu Ser Cys Lys Cys Asn Gly Trp Lys
            100                 105                 110
Asn Pro Asn Pro Ser Pro Thr Pro Pro Arg Ala Asp Leu Gln Gln Ile
        115                 120                 125
Ile Val Ser Leu Thr Glu Ser Cys Arg Ser Cys Ser His Ala Leu Ala
    130                 135                 140
Ala His Val Ser His Leu Glu Asn Val Ser Glu Glu Glu Met Asn Arg
145                 150                 155                 160
Leu Leu Gly Ile Val Leu Asp Val Glu Tyr Leu Phe Thr Cys Val His
                165                 170                 175
Lys Glu Glu Asp Ala Asp Thr Lys Gln Val Tyr Phe Tyr Leu Phe Lys
            180                 185                 190
Leu Leu Arg Lys Ser Ile Leu Gln Arg Gly Lys Pro Val Val Glu Gly
        195                 200                 205
Ser Leu Glu Lys Lys Pro Pro Phe Glu Lys Pro Ser Ile Glu Gln Gly
    210                 215                 220
Val Asn Asn Phe Val Gln Tyr Lys Phe Ser His Leu Pro Ala Lys Glu
225                 230                 235                 240
Arg Gln Thr Ile Val Glu Leu Ala Lys Met Phe Leu Asn Arg Ile Asn
                245                 250                 255
Tyr Trp His Leu Glu Ala Pro Ser Gln Arg Arg Leu Arg Ser Pro Asn
            260                 265                 270
Asp Asp Ile Ser Gly Tyr Lys Glu Asn Tyr Thr Arg Trp Leu Cys Tyr
        275                 280                 285
Cys Asn Val Pro Gln Phe Cys Asp Ser Leu Pro Arg Tyr Glu Thr Thr
    290                 295                 300
Gln Val Phe Gly Arg Thr Leu Leu Arg Ser Val Phe Thr Val Met Arg
305                 310                 315                 320
Arg Gln Leu Leu Glu Gln Ala Arg Gln Glu Lys Asp Lys Leu Pro Leu
                325                 330                 335
Glu Lys Arg Thr Leu Ile Leu Thr His Phe Pro Lys Phe Leu Ser Met
            340                 345                 350
Leu Glu Glu Glu Val Tyr Ser Gln Asn Ser Pro Ile Trp Asp Gln Asp
        355                 360                 365
Phe Leu Ser Ala Ser Ser Arg Thr Ser Gln Leu Gly Ile Gln Thr Val
    370                 375                 380
Ile Asn Pro Pro Pro Val Ala Gly Thr Ile Ser Tyr Asn Ser Thr Ser
385                 390                 395                 400
Ser Ser Leu Glu Gln Pro Asn Ala Gly Ser Ser Ser Pro Ala Cys Lys
                405                 410                 415
Ala Ser Ser Gly Leu Glu Ala Asn Pro Gly Glu Lys Arg Lys Met Thr
            420                 425                 430
Asp Ser His Val Leu Glu Glu Ala Lys Lys Pro Arg Val Met Gly Asp
        435                 440                 445
Ile Pro Met Glu Leu Ile Asn Glu Val Met Ser Thr Ile Thr Asp Pro
    450                 455                 460
Ala Ala Met Leu Gly Pro Glu Thr Asn Phe Leu Ser Ala His Ser Ala
465                 470                 475                 480
```

```
Arg Asp Glu Ala Ala Arg Leu Glu Glu Arg Arg Gly Val Ile Glu Phe
                485                 490                 495
His Val Val Gly Asn Ser Leu Asn Gln Lys Pro Asn Lys Lys Ile Leu
            500                 505                 510
Met Trp Leu Val Gly Leu Gln Asn Val Phe Ser His Gln Leu Pro Arg
        515                 520                 525
Met Pro Lys Glu Tyr Ile Thr Arg Leu Val Phe Asp Pro Lys His Lys
    530                 535                 540
Thr Leu Ala Leu Ile Lys Asp Gly Arg Val Ile Gly Gly Ile Cys Phe
545                 550                 555                 560
Arg Met Phe Pro Ser Gln Gly Phe Thr Glu Ile Val Phe Cys Ala Val
                565                 570                 575
Thr Ser Asn Glu Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn His
            580                 585                 590
Leu Lys Glu Tyr His Ile Lys His Asp Ile Leu Asn Phe Leu Thr Tyr
        595                 600                 605
Ala Asp Glu Tyr Ala Ile Gly Tyr Phe Lys Lys Gln Gly Phe Ser Lys
    610                 615                 620
Glu Ile Lys Ile Pro Lys Thr Lys Tyr Val Gly Tyr Ile Lys Asp Tyr
625                 630                 635                 640
Glu Gly Ala Thr Leu Met Gly Cys Glu Leu Asn Pro Arg Ile Pro Tyr
                645                 650                 655
Thr Glu Phe Ser Val Ile Ile Lys Lys Gln Lys Glu Ile Ile Lys Lys
            660                 665                 670
Leu Ile Glu Arg Lys Gln Ala Gln Ile Arg Lys Val Tyr Pro Gly Leu
        675                 680                 685
Ser Cys Phe Lys Asp Gly Val Arg Gln Ile Pro Ile Glu Ser Ile Pro
    690                 695                 700
Gly Ile Arg Glu Thr Gly Trp Lys Pro Ser Gly Lys Glu Lys Ser Lys
705                 710                 715                 720
Glu Pro Arg Asp Pro Asp Gln Leu Tyr Ser Thr Leu Lys Ser Ile Leu
                725                 730                 735
Gln Gln Val Lys Ser His Gln Ser Ala Trp Pro Phe Met Glu Pro Val
            740                 745                 750
Lys Arg Thr Glu Ala Pro Gly Tyr Tyr Glu Val Ile Arg Ser Pro Met
        755                 760                 765
Asp Leu Lys Thr Met Ser Glu Arg Leu Lys Asn Arg Tyr Tyr Val Ser
    770                 775                 780
Lys Lys Leu Phe Met Ala Asp Leu Gln Arg Val Phe Thr Asn Cys Lys
785                 790                 795                 800
Glu Tyr Asn Ala Pro Glu Ser Glu Tyr Tyr Lys Cys Ala Asn Ile Leu
                805                 810                 815
Glu Lys Phe Phe Phe Ser Lys Ile Lys Glu Ala Gly Leu Ile Asp Lys
            820                 825                 830
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 481 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Leu Glu Glu Glu Val Tyr Ser Gln Asn Ser Pro Ile Trp Asp Gln
 1               5                  10                  15
Asp Phe Leu Ser Ala Ser Ser Arg Thr Ser Gln Leu Gly Ile Gln Thr
            20                  25                  30
Val Ile Asn Pro Pro Pro Val Ala Gly Thr Ile Ser Tyr Asn Ser Thr
        35                  40                  45
```

```
Ser Ser Ser Leu Glu Gln Pro Asn Ala Gly Ser Ser Ser Pro Ala Cys
    50                  55                  60
Lys Ala Ser Ser Gly Leu Glu Ala Asn Pro Gly Glu Lys Arg Lys Met
65                  70                  75                  80
Thr Asp Ser His Val Leu Glu Glu Ala Lys Lys Pro Arg Val Met Gly
                85                  90                  95
Asp Ile Pro Met Glu Leu Ile Asn Glu Val Met Ser Thr Ile Thr Asp
            100                 105                 110
Pro Ala Ala Met Leu Gly Pro Glu Thr Asn Phe Leu Ser Ala His Ser
        115                 120                 125
Ala Arg Asp Glu Ala Ala Arg Leu Glu Glu Arg Arg Gly Val Ile Glu
    130                 135                 140
Phe His Val Val Gly Asn Ser Leu Asn Gln Lys Pro Asn Lys Lys Ile
145                 150                 155                 160
Leu Met Trp Leu Val Gly Leu Gln Asn Val Phe Ser His Gln Leu Pro
                165                 170                 175
Arg Met Pro Lys Glu Tyr Ile Thr Arg Leu Val Phe Asp Pro Lys His
            180                 185                 190
Lys Thr Leu Ala Leu Ile Lys Asp Gly Arg Val Ile Gly Gly Ile Cys
        195                 200                 205
Phe Arg Met Phe Pro Ser Gln Gly Phe Thr Glu Ile Val Phe Cys Ala
    210                 215                 220
Val Thr Ser Asn Glu Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn
225                 230                 235                 240
His Leu Lys Glu Tyr His Ile Lys His Asp Ile Leu Asn Phe Leu Thr
                245                 250                 255
Tyr Ala Asp Glu Tyr Ala Ile Gly Tyr Phe Lys Lys Gln Gly Phe Ser
            260                 265                 270
Lys Glu Ile Lys Ile Pro Lys Thr Lys Tyr Val Gly Tyr Ile Lys Asp
        275                 280                 285
Tyr Glu Gly Ala Thr Leu Met Gly Cys Glu Leu Asn Pro Arg Ile Pro
    290                 295                 300
Tyr Thr Glu Phe Ser Val Ile Ile Lys Lys Gln Lys Glu Ile Ile Lys
305                 310                 315                 320
Lys Leu Ile Glu Arg Lys Gln Ala Gln Ile Arg Lys Val Tyr Pro Gly
                325                 330                 335
Leu Ser Cys Phe Lys Asp Gly Val Arg Gln Ile Pro Ile Glu Ser Ile
            340                 345                 350
Pro Gly Ile Arg Glu Thr Gly Trp Lys Pro Ser Gly Lys Glu Lys Ser
        355                 360                 365
Lys Glu Pro Arg Asp Pro Asp Gln Leu Tyr Ser Thr Leu Lys Ser Ile
    370                 375                 380
Leu Gln Gln Val Lys Ser His Gln Ser Ala Trp Pro Phe Met Glu Pro
385                 390                 395                 400
Val Lys Arg Thr Glu Ala Pro Gly Tyr Tyr Glu Val Ile Arg Ser Pro
                405                 410                 415
Met Asp Leu Lys Thr Met Ser Glu Arg Leu Lys Asn Arg Tyr Tyr Val
            420                 425                 430
Ser Lys Lys Leu Phe Met Ala Asp Leu Gln Arg Val Phe Thr Asn Cys
        435                 440                 445
Lys Glu Tyr Asn Ala Pro Glu Ser Glu Tyr Tyr Lys Cys Ala Asn Ile
    450                 455                 460
Leu Glu Lys Phe Phe Phe Ser Lys Ile Lys Glu Ala Gly Leu Ile Asp
465                 470                 475                 480
Lys
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 203 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Arg Val Val Gln His Thr Lys Gly Cys Lys Arg Lys Thr Asn Gly Gly
 1               5                  10                  15
Cys Pro Ile Cys Lys Gln Leu Ile Ala Leu Cys Cys Tyr His Ala Lys
            20                  25                  30
His Cys Gln Glu Asn Lys Cys Pro Val Pro Phe Cys Leu Asn Ile Lys
        35                  40                  45
Gln Lys Leu Arg Gln Gln Gln Leu Gln His Arg Leu Gln Gln Ala Gln
    50                  55                  60
Met Leu Arg Arg Arg Met Ala Ser Met Arg Thr Gly Val Val Gly Gln
65                  70                  75                  80
Gln Gln Gly Leu Pro Ser Pro Thr Pro Ala Thr Pro Thr Thr Pro Thr
                85                  90                  95
Gly Gln Gln Pro Thr Thr Pro Gln Thr Pro Gln Pro Thr Ser Gln Pro
            100                 105                 110
Gln Pro Thr Pro Pro Asn Ser Met Pro Pro Tyr Leu Pro Arg Thr Gln
        115                 120                 125
Ala Ala Gly Pro Val Ser Gln Gly Lys Ala Ala Gly Gln Val Thr Pro
    130                 135                 140
Pro Thr Pro Pro Gln Thr Ala Gln Pro Pro Leu Pro Gly Pro Pro Pro
145                 150                 155                 160
Thr Ala Val Glu Met Ala Met Gln Ile Gln Arg Ala Ala Glu Thr Gln
                165                 170                 175
Arg Gln Met Ala His Val Gln Ile Phe Gln Arg Pro Ile Gln His Gln
            180                 185                 190
Met Pro Pro Met Thr Pro Met Ala Pro Met Gly
        195                 200
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Glu Ala Gly Gly Ala Gly Pro Gly Gly Cys Gly Ala Gly Ala
 1               5                  10                  15
Gly Ala Gly Ala Gly Pro Gly Ala Leu Pro Pro Gln Pro Ala Ala Leu
            20                  25                  30
Pro Pro Ala Pro Pro Gln Gly Ser Pro Cys Ala Ala Ala Ala Gly Gly
        35                  40                  45
Ser Gly Ala Cys Gly Pro Ala Thr Ala Val Ala Ala Ala Gly Thr Ala
    50                  55                  60
Glu Gly Pro Gly Gly Gly Gly Ser Ala Arg Ile Ala Val Lys Lys Ala
65                  70                  75                  80
Gln Leu Arg Ser Ala Pro Arg Ala Lys Lys Leu Glu Lys Leu Gly Val
                85                  90                  95
Tyr Ser Ala Cys Lys Ala Glu Glu Ser Cys Lys Cys Asn Gly Trp Lys
            100                 105                 110
Asn Pro Asn Pro Ser Pro Thr Pro Pro Arg Ala Asp Leu Gln Gln Ile
        115                 120                 125
Ile Val Ser Leu Thr Glu Ser Cys Arg Ser Cys Ser His Ala Leu Ala
    130                 135                 140
```

```
Ala His Val Ser His Leu Glu Asn Val Ser Glu Glu Glu Met Asn Arg
145                 150                 155                 160
Leu Leu Gly Ile Val Leu Asp Val Glu Tyr Leu Phe Thr Cys Val His
                165                 170                 175
Lys Glu Glu Asp Ala Asp Thr Lys Gln Val Tyr Phe Tyr Leu Phe Lys
            180                 185                 190
Leu Leu Arg Lys Ser Ile Leu Gln Arg Gly Lys Pro Val Val Glu Gly
        195                 200                 205
Ser Leu Glu Lys Lys Pro Pro Phe Glu Lys Pro Ser Ile Glu Gln Gly
    210                 215                 220
Val Asn Asn Phe Val Gln Tyr Lys Phe Ser His Leu Pro Ala Lys Glu
225                 230                 235                 240
Arg Gln Thr Ile Val Glu Leu Ala Lys Met Phe Leu Asn Arg Ile Asn
                245                 250                 255
Tyr Trp His Leu Glu Ala Pro Ser Gln Arg Arg Leu Arg Ser Pro Asn
            260                 265                 270
Asp Asp Ile Ser Gly Tyr Lys Glu Asn Tyr Thr Arg Trp Leu Cys Tyr
        275                 280                 285
Cys Asn Val Pro Gln Phe Cys Asp Ser Leu Pro Arg Tyr Glu Thr Thr
    290                 295                 300
Gln Val Phe Gly Arg Thr Leu Leu Arg Ser Val Phe Thr Val Met Arg
305                 310                 315                 320
Arg Gln Leu Leu Glu Gln Ala Arg Gln Glu Lys Asp Lys Leu Pro Leu
                325                 330                 335
Glu Lys Arg Thr Leu Ile Leu Thr His Phe Pro Lys Phe Leu Ser
            340                 345                 350
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 476 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Leu Glu Glu Glu Ile Tyr Gly Ala Asn Ser Pro Ile Trp Glu Ser
 1               5                  10                  15
Gly Phe Thr Met Pro Pro Ser Glu Gly Thr Gln Leu Val Pro Arg Pro
            20                  25                  30
Ala Ser Val Ser Ala Ala Val Val Pro Ser Thr Pro Ile Phe Ser Pro
        35                  40                  45
Ser Met Gly Gly Gly Ser Asn Ser Ser Leu Ser Leu Asp Ser Ala Gly
    50                  55                  60
Ala Glu Pro Met Pro Gly Glu Lys Arg Thr Leu Pro Glu Asn Leu Thr
65                  70                  75                  80
Leu Glu Asp Ala Lys Arg Leu Arg Val Met Gly Asp Ile Pro Met Glu
                85                  90                  95
Leu Val Asn Glu Val Met Leu Thr Ile Thr Asp Pro Ala Ala Met Leu
            100                 105                 110
Gly Pro Glu Thr Ser Leu Leu Ser Ala Asn Ala Ala Arg Asp Glu Thr
        115                 120                 125
Ala Arg Leu Glu Glu Arg Arg Gly Ile Ile Glu Phe His Val Ile Gly
    130                 135                 140
Asn Ser Leu Thr Pro Lys Ala Asn Arg Arg Val Leu Leu Trp Leu Val
145                 150                 155                 160
Gly Leu Gln Asn Val Phe Ser His Gln Leu Pro Arg Met Pro Lys Glu
                165                 170                 175
Tyr Ile Ala Arg Leu Val Phe Asp Pro Lys His Lys Thr Leu Ala Leu
            180                 185                 190
```

```
Ile Lys Asp Gly Arg Val Ile Gly Gly Ile Cys Phe Arg Met Phe Pro
        195                 200                 205
Thr Gln Gly Phe Thr Glu Ile Val Phe Cys Ala Val Thr Ser Asn Glu
    210                 215                 220
Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn His Leu Lys Glu Tyr
225                 230                 235                 240
His Ile Lys His Asn Ile Leu Tyr Phe Leu Thr Tyr Ala Asp Glu Tyr
                245                 250                 255
Ala Ile Gly Tyr Phe Lys Lys Gln Gly Phe Ser Lys Asp Ile Lys Val
            260                 265                 270
Pro Lys Ser Arg Tyr Leu Gly Tyr Ile Lys Asp Tyr Glu Gly Ala Thr
        275                 280                 285
Leu Met Glu Cys Glu Leu Asn Pro Arg Ile Pro Tyr Thr Glu Leu Ser
    290                 295                 300
His Ile Ile Lys Lys Gln Lys Glu Ile Ile Lys Lys Leu Ile Glu Arg
305                 310                 315                 320
Lys Gln Ala Gln Ile Arg Lys Val Tyr Pro Gly Leu Ser Cys Phe Lys
                325                 330                 335
Glu Gly Val Arg Gln Ile Pro Val Glu Ser Val Pro Gly Ile Arg Glu
            340                 345                 350
Thr Gly Trp Lys Pro Leu Gly Lys Glu Lys Gly Lys Glu Leu Lys Asp
        355                 360                 365
Pro Asp Gln Leu Tyr Thr Thr Leu Lys Asn Leu Leu Ala Gln Ile Lys
    370                 375                 380
Ser His Pro Ser Ala Trp Pro Phe Met Glu Pro Val Lys Lys Ser Glu
385                 390                 395                 400
Ala Pro Asp Tyr Tyr Glu Val Ile Arg Phe Pro Ile Asp Leu Lys Thr
                405                 410                 415
Met Thr Glu Arg Leu Arg Ser Arg Tyr Tyr Val Thr Arg Lys Leu Phe
            420                 425                 430
Val Ala Asp Leu Gln Arg Val Ile Ala Asn Cys Arg Glu Tyr Asn Pro
        435                 440                 445
Pro Asp Ser Glu Tyr Cys Arg Cys Ala Ser Ala Leu Glu Lys Phe Phe
    450                 455                 460
Tyr Phe Lys Leu Lys Glu Gly Gly Leu Ile Asp Lys
465                 470                 475
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2414 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Glu Asn Val Val Glu Pro Gly Pro Pro Ser Ala Lys Arg Pro
 1               5                  10                  15
Lys Leu Ser Ser Pro Ala Leu Ser Ala Ser Ala Ser Asp Gly Thr Asp
            20                  25                  30
Phe Gly Ser Leu Phe Asp Leu Glu His Asp Leu Pro Asp Glu Leu Ile
        35                  40                  45
Asn Ser Thr Glu Leu Gly Leu Thr Asn Gly Gly Asp Ile Asn Gln Leu
    50                  55                  60
Gln Thr Ser Leu Gly Met Val Gln Asp Ala Ala Ser Lys His Lys Gln
65                  70                  75                  80
Leu Ser Glu Leu Leu Arg Ser Gly Ser Ser Pro Asn Leu Asn Met Gly
                85                  90                  95
Val Gly Gly Pro Gly Gln Val Met Ala Ser Gln Ala Gln Gln Ser Ser
            100                 105                 110
```

```
Pro Gly Leu Gly Leu Ile Asn Ser Met Val Lys Ser Pro Met Thr Gln
        115                 120                 125
Ala Gly Leu Thr Ser Pro Asn Met Gly Met Gly Thr Ser Gly Pro Asn
    130                 135                 140
Gln Gly Pro Thr Gln Ser Thr Gly Met Met Asn Ser Pro Val Asn Gln
145                 150                 155                 160
Pro Ala Met Gly Met Asn Thr Gly Thr Asn Ala Gly Met Asn Pro Gly
                165                 170                 175
Met Leu Ala Ala Gly Asn Gly Gln Gly Ile Met Pro Asn Gln Val Met
            180                 185                 190
Asn Gly Ser Ile Gly Ala Gly Arg Gly Arg Gln Asp Met Gln Tyr Pro
        195                 200                 205
Asn Pro Gly Met Gly Ser Ala Gly Asn Leu Leu Thr Glu Pro Leu Gln
    210                 215                 220
Gln Gly Ser Pro Gln Met Gly Gly Gln Thr Gly Leu Arg Gly Pro Gln
225                 230                 235                 240
Pro Leu Lys Met Gly Met Met Asn Asn Pro Asn Pro Tyr Gly Ser Pro
                245                 250                 255
Tyr Thr Gln Asn Pro Gly Gln Gln Ile Gly Ala Ser Gly Leu Gly Leu
            260                 265                 270
Gln Ile Gln Thr Lys Thr Val Leu Ser Asn Asn Leu Ser Pro Phe Ala
        275                 280                 285
Met Asp Lys Lys Ala Val Pro Gly Gly Gly Met Pro Asn Met Gly Gln
    290                 295                 300
Gln Pro Ala Pro Gln Val Gln Gln Pro Gly Leu Val Thr Pro Val Ala
305                 310                 315                 320
Gln Gly Met Gly Ser Gly Ala His Thr Ala Asp Pro Glu Lys Arg Lys
                325                 330                 335
Leu Ile Gln Gln Gln Leu Val Leu Leu Leu His Ala His Lys Cys Gln
            340                 345                 350
Arg Arg Glu Gln Ala Asn Gly Glu Val Arg Gln Cys Asn Leu Pro His
        355                 360                 365
Cys Arg Thr Met Lys Asn Val Leu Asn His Met Thr His Cys Gln Ser
    370                 375                 380
Gly Lys Ser Cys Gln Val Ala His Cys Ala Ser Ser Arg Gln Ile Ile
385                 390                 395                 400
Ser His Trp Lys Asn Cys Thr Arg His Asp Cys Pro Val Cys Leu Pro
                405                 410                 415
Leu Lys Asn Ala Gly Asp Lys Arg Asn Gln Gln Pro Ile Leu Thr Gly
            420                 425                 430
Ala Pro Val Gly Leu Gly Asn Pro Ser Ser Leu Gly Val Gly Gln Gln
        435                 440                 445
Ser Ala Pro Asn Leu Ser Thr Val Ser Gln Ile Asp Pro Ser Ser Ile
    450                 455                 460
Glu Arg Ala Tyr Ala Ala Leu Gly Leu Pro Tyr Gln Val Asn Gln Met
465                 470                 475                 480
Pro Thr Gln Pro Gln Val Gln Ala Lys Asn Gln Gln Asn Gln Gln Pro
                485                 490                 495
Gly Gln Ser Pro Gln Gly Met Arg Pro Met Ser Asn Met Ser Ala Ser
            500                 505                 510
Pro Met Gly Val Asn Gly Gly Val Gly Val Gln Thr Pro Ser Leu Leu
        515                 520                 525
Ser Asp Ser Met Leu His Ser Ala Ile Asn Ser Gln Asn Pro Met Met
    530                 535                 540
Ser Glu Asn Ala Ser Val Pro Ser Leu Gly Pro Met Pro Thr Ala Ala
545                 550                 555                 560
Gln Pro Ser Thr Thr Gly Ile Arg Lys Gln Trp His Glu Asp Ile Thr
                565                 570                 575
Gln Asp Leu Arg Asn His Leu Val His Lys Leu Val Gln Ala Ile Phe
            580                 585                 590
Pro Thr Pro Asp Pro Ala Ala Leu Lys Asp Arg Arg Met Glu Asn Leu
        595                 600                 605
```

```
Val Ala Tyr Ala Arg Lys Val Glu Gly Asp Met Tyr Glu Ser Ala Asn
    610                 615                 620
Asn Arg Ala Glu Tyr Tyr His Leu Leu Ala Glu Lys Ile Tyr Lys Ile
625                 630                 635                 640
Gln Lys Glu Leu Glu Glu Lys Arg Arg Thr Arg Leu Gln Lys Gln Asn
                645                 650                 655
Met Leu Pro Asn Ala Ala Gly Met Val Pro Val Ser Met Asn Pro Gly
            660                 665                 670
Pro Asn Met Gly Gln Pro Gln Pro Gly Met Thr Ser Asn Gly Pro Leu
        675                 680                 685
Pro Asp Pro Ser Met Ile Arg Gly Ser Val Pro Asn Gln Met Met Pro
    690                 695                 700
Arg Ile Thr Pro Gln Ser Gly Leu Asn Gln Phe Gly Gln Met Ser Met
705                 710                 715                 720
Ala Gln Pro Pro Ile Val Pro Arg Gln Thr Pro Pro Leu Gln His His
                725                 730                 735
Gly Gln Leu Ala Gln Pro Gly Ala Leu Asn Pro Pro Met Gly Tyr Gly
            740                 745                 750
Pro Arg Met Gln Gln Pro Ser Asn Gln Gly Gln Phe Leu Pro Gln Thr
        755                 760                 765
Gln Phe Pro Ser Gln Gly Met Asn Val Thr Asn Ile Pro Leu Ala Pro
    770                 775                 780
Ser Ser Gly Gln Ala Pro Val Ser Gln Ala Gln Met Ser Ser Ser Ser
785                 790                 795                 800
Cys Pro Val Asn Ser Pro Ile Met Pro Pro Gly Ser Gln Gly Ser His
                805                 810                 815
Ile His Cys Pro Gln Leu Pro Gln Pro Ala Leu His Gln Asn Ser Pro
            820                 825                 830
Ser Pro Val Pro Ser Arg Thr Pro Thr Pro His His Thr Pro Pro Ser
        835                 840                 845
Ile Gly Ala Gln Gln Pro Pro Ala Thr Thr Ile Pro Ala Pro Val Pro
    850                 855                 860
Thr Pro Pro Ala Met Pro Pro Gly Pro Gln Ser Gln Ala Leu His Pro
865                 870                 875                 880
Pro Pro Arg Gln Thr Pro Thr Pro Pro Thr Thr Gln Leu Pro Gln Gln
                885                 890                 895
Val Gln Pro Ser Leu Pro Ala Ala Pro Ser Ala Asp Gln Pro Gln Gln
            900                 905                 910
Gln Pro Arg Ser Gln Gln Ser Thr Ala Ala Ser Val Pro Thr Pro Asn
        915                 920                 925
Ala Pro Leu Leu Pro Pro Gln Pro Ala Thr Pro Leu Ser Gln Pro Ala
    930                 935                 940
Val Ser Ile Glu Gly Gln Val Ser Asn Pro Pro Ser Thr Ser Ser Thr
945                 950                 955                 960
Glu Val Asn Ser Gln Ala Ile Ala Glu Lys Gln Pro Ser Gln Glu Val
                965                 970                 975
Lys Met Glu Ala Lys Met Glu Val Asp Gln Pro Glu Pro Ala Asp Thr
            980                 985                 990
Gln Pro Glu Asp Ile Ser Glu Ser Lys Val Glu Asp Cys Lys Met Glu
        995                1000                1005
Ser Thr Glu Thr Glu Glu Arg Ser Thr Glu Leu Lys Thr Glu Ile Lys
   1010                1015                1020
Glu Glu Glu Asp Gln Pro Ser Thr Ser Ala Thr Gln Ser Ser Pro Ala
025                1030                1035                1040
Pro Gly Gln Ser Lys Lys Lys Ile Phe Lys Pro Glu Glu Leu Arg Gln
               1045                1050                1055
Ala Leu Met Pro Thr Leu Glu Ala Leu Tyr Arg Gln Asp Pro Glu Ser
           1060                1065                1070
Leu Pro Phe Arg Gln Pro Val Asp Pro Gln Leu Leu Gly Ile Pro Asp
       1075                1080                1085
Tyr Phe Asp Ile Val Lys Ser Pro Met Asp Leu Ser Thr Ile Lys Arg
   1090                1095                1100
```

Lys Leu Asp Thr Gly Gln Tyr Gln Glu Pro Trp Gln Tyr Val Asp Asp
105 1110 1115 1120
Ile Trp Leu Met Phe Asn Asn Ala Trp Leu Tyr Asn Arg Lys Thr Ser
1125 1130 1135
Arg Val Tyr Lys Tyr Cys Ser Lys Leu Ser Glu Val Phe Glu Gln Glu
1140 1145 1150
Ile Asp Pro Val Met Gln Ser Leu Gly Tyr Cys Cys Gly Arg Lys Leu
1155 1160 1165
Glu Phe Ser Pro Gln Thr Leu Cys Cys Tyr Gly Lys Gln Leu Cys Thr
1170 1175 1180
Ile Pro Arg Asp Ala Thr Tyr Tyr Ser Tyr Gln Asn Arg Tyr His Phe
185 1190 1195 1200
Cys Glu Lys Cys Phe Asn Glu Ile Gln Gly Glu Ser Val Ser Leu Gly
1205 1210 1215
Asp Asp Pro Ser Gln Pro Gln Thr Thr Ile Asn Lys Glu Gln Phe Ser
1220 1225 1230
Lys Arg Lys Asn Asp Thr Leu Asp Pro Glu Leu Phe Val Glu Cys Thr
1235 1240 1245
Glu Cys Gly Arg Lys Met His Gln Ile Cys Val Leu His His Glu Ile
1250 1255 1260
Ile Trp Pro Ala Gly Phe Val Cys Asp Gly Cys Leu Lys Lys Ser Ala
265 1270 1275 1280
Arg Thr Arg Lys Glu Asn Lys Phe Ser Ala Lys Arg Leu Pro Ser Thr
1285 1290 1295
Arg Leu Gly Thr Phe Leu Glu Asn Arg Val Asn Asp Phe Leu Arg Arg
1300 1305 1310
Gln Asn His Pro Glu Ser Gly Glu Val Thr Val Arg Val Val His Ala
1315 1320 1325
Ser Asp Lys Thr Val Glu Val Lys Pro Gly Met Lys Ala Arg Phe Val
1330 1335 1340
Asp Ser Gly Glu Met Ala Glu Ser Phe Pro Tyr Arg Thr Lys Ala Leu
345 1350 1355 1360
Phe Ala Phe Glu Glu Ile Asp Gly Val Asp Leu Cys Phe Phe Gly Met
1365 1370 1375
His Val Gln Glu Tyr Gly Ser Asp Cys Pro Pro Pro Asn Gln Arg Arg
1380 1385 1390
Val Tyr Ile Ser Tyr Leu Asp Ser Val His Phe Phe Arg Pro Lys Cys
1395 1400 1405
Leu Arg Thr Ala Val Tyr His Glu Ile Leu Ile Gly Tyr Leu Glu Tyr
1410 1415 1420
Val Lys Lys Leu Gly Tyr Thr Thr Gly His Ile Trp Ala Cys Pro Pro
425 1430 1435 1440
Ser Glu Gly Asp Asp Tyr Ile Phe His Cys His Pro Pro Asp Gln Lys
1445 1450 1455
Ile Pro Lys Pro Lys Arg Leu Gln Glu Trp Tyr Lys Lys Met Leu Asp
1460 1465 1470
Lys Ala Val Ser Glu Arg Ile Val His Asp Tyr Lys Asp Ile Phe Lys
1475 1480 1485
Gln Ala Thr Glu Asp Arg Leu Thr Ser Ala Lys Glu Leu Pro Tyr Phe
1490 1495 1500
Glu Gly Asp Phe Trp Pro Asn Val Leu Glu Glu Ser Ile Lys Glu Leu
505 1510 1515 1520
Glu Gln Glu Glu Glu Glu Arg Lys Arg Glu Glu Asn Thr Ser Asn Glu
1525 1530 1535
Ser Thr Asp Val Thr Lys Gly Asp Ser Lys Asn Ala Lys Lys Lys Asn
1540 1545 1550
Asn Lys Lys Thr Ser Lys Asn Lys Ser Ser Leu Ser Arg Gly Asn Lys
1555 1560 1565
Lys Lys Pro Gly Met Pro Asn Val Ser Asn Asp Leu Ser Gln Lys Leu
1570 1575 1580
Tyr Ala Thr Met Glu Lys His Lys Glu Val Phe Phe Val Ile Arg Leu
585 1590 1595 1600

Ile Ala Gly Pro Ala Ala Asn Ser Leu Pro Pro Ile Val Asp Pro Asp
1605 1610 1615
Pro Leu Ile Pro Cys Asp Leu Met Asp Gly Arg Asp Ala Phe Leu Thr
1620 1625 1630
Leu Ala Arg Asp Lys His Leu Glu Phe Ser Ser Leu Arg Arg Ala Gln
1635 1640 1645
Trp Ser Thr Met Cys Met Leu Val Glu Leu His Thr Gln Ser Gln Asp
1650 1655 1660
Arg Phe Val Tyr Thr Cys Asn Glu Cys Lys His His Val Glu Thr Arg
665 1670 1675 1680
Trp His Cys Thr Val Cys Glu Asp Tyr Asp Leu Cys Ile Thr Cys Tyr
1685 1690 1695
Asn Thr Lys Asn His Asp His Lys Met Glu Lys Leu Gly Leu Gly Leu
1700 1705 1710
Asp Asp Glu Ser Asn Asn Gln Gln Ala Ala Ala Thr Gln Ser Pro Gly
1715 1720 1725
Asp Ser Arg Arg Leu Ser Ile Gln Arg Cys Ile Gln Ser Leu Val His
1730 1735 1740
Ala Cys Gln Cys Arg Asn Ala Asn Cys Ser Leu Pro Ser Cys Gln Lys
745 1750 1755 1760
Met Lys Arg Val Val Gln His Thr Lys Gly Cys Lys Arg Lys Thr Asn
1765 1770 1775
Gly Gly Cys Pro Ile Cys Lys Gln Leu Ile Ala Leu Cys Cys Tyr His
1780 1785 1790
Ala Lys His Cys Gln Glu Asn Lys Cys Pro Val Pro Phe Cys Leu Asn
1795 1800 1805
Ile Lys Gln Lys Leu Arg Gln Gln Gln Leu Gln His Arg Leu Gln Gln
1810 1815 1820
Ala Gln Met Leu Arg Arg Arg Met Ala Ser Met Gln Arg Thr Gly Val
825 1830 1835 1840
Val Gly Gln Gln Gln Gly Leu Pro Ser Pro Thr Pro Ala Thr Pro Thr
1845 1850 1855
Thr Pro Thr Gly Gln Gln Pro Thr Thr Pro Gln Thr Pro Gln Pro Thr
1860 1865 1870
Ser Gln Pro Gln Pro Thr Pro Pro Asn Ser Met Pro Pro Tyr Leu Pro
1875 1880 1885
Arg Thr Gln Ala Ala Gly Pro Val Ser Gln Gly Lys Ala Ala Gly Gln
1890 1895 1900
Val Thr Pro Pro Thr Pro Pro Gln Thr Ala Gln Pro Pro Leu Pro Gly
905 1910 1915 1920
Pro Pro Pro Thr Ala Val Glu Met Ala Met Gln Ile Gln Arg Ala Ala
1925 1930 1935
Glu Thr Gln Arg Gln Met Ala His Val Gln Ile Phe Gln Arg Pro Ile
1940 1945 1950
Gln His Gln Met Pro Pro Met Thr Pro Met Ala Pro Met Gly Met Asn
1955 1960 1965
Pro Pro Pro Met Thr Arg Gly Pro Ser Gly His Leu Glu Pro Gly Met
1970 1975 1980
Gly Pro Thr Gly Met Gln Gln Gln Pro Pro Trp Ser Gln Gly Gly Leu
985 1990 1995 2000
Pro Gln Pro Gln Gln Leu Gln Ser Gly Met Pro Arg Pro Ala Met Met
2005 2010 2015
Ser Val Ala Gln His Gly Gln Pro Leu Asn Met Ala Pro Gln Pro Gly
2020 2025 2030
Leu Gly Gln Val Gly Ile Ser Pro Leu Lys Pro Gly Thr Val Ser Gln
2035 2040 2045
Gln Ala Leu Gln Asn Leu Leu Arg Thr Leu Arg Ser Pro Ser Ser Pro
2050 2055 2060
Leu Gln Gln Gln Gln Val Leu Ser Ile Leu His Ala Asn Pro Gln Leu
065 2070 2075 2080
Leu Ala Ala Phe Ile Lys Gln Arg Ala Ala Lys Tyr Ala Asn Ser Asn
2085 2090 2095

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Pro Gln Pro Ile Pro Gly Gln Pro Gly Met Pro Gln Gly Gln Pro Gly
          2100                2105                2110
Leu Gln Pro Pro Thr Met Pro Gly Gln Gln Gly Val His Ser Asn Pro
       2115                2120                2125
Ala Met Gln Asn Met Asn Pro Met Gln Ala Gly Val Gln Arg Ala Gly
   2130                2135                2140
Leu Pro Gln Gln Gln Pro Gln Gln Gln Leu Gln Pro Pro Met Gly Gly
145                2150                2155                2160
Met Ser Pro Gln Ala Gln Gln Met Asn Met Asn His Asn Thr Met Pro
               2165                2170                2175
Ser Gln Phe Arg Asp Ile Leu Arg Arg Gln Gln Met Met Gln Gln Gln
          2180                2185                2190
Gln Gln Gln Gly Ala Gly Pro Gly Ile Gly Pro Gly Met Ala Asn His
       2195                2200                2205
Asn Gln Phe Gln Gln Pro Gln Gly Val Gly Tyr Pro Pro Gln Pro Gln
   2210                2215                2220
Gln Arg Met Gln His His Met Gln Gln Met Gln Gln Gly Asn Met Gly
225                2230                2235                2240
Gln Ile Gly Gln Leu Pro Gln Ala Leu Gly Ala Glu Ala Gly Ala Ser
               2245                2250                2255
Leu Gln Ala Tyr Gln Gln Arg Leu Leu Gln Gln Gln Met Gly Ser Pro
          2260                2265                2270
Val Gln Pro Asn Pro Met Ser Pro Gln Gln His Met Leu Pro Asn Gln
       2275                2280                2285
Ala Gln Ser Pro His Leu Gln Gly Gln Gln Ile Pro Asn Ser Leu Ser
   2290                2295                2300
Asn Gln Val Arg Ser Pro Gln Pro Val Pro Ser Pro Arg Pro Gln Ser
305                2310                2315                2320
Gln Pro Pro His Ser Ser Pro Ser Pro Arg Met Gln Pro Gln Pro Ser
               2325                2330                2335
Pro His His Val Ser Pro Gln Thr Ser Ser Pro His Pro Gly Leu Val
          2340                2345                2350
Ala Ala Gln Ala Asn Pro Met Glu Gln Gly His Phe Ala Ser Pro Asp
       2355                2360                2365
Gln Asn Ser Met Leu Ser Gln Leu Ala Ser Asn Pro Gly Met Ala Asn
   2370                2375                2380
Leu His Gly Ala Ser Ala Thr Asp Leu Gly Leu Ser Thr Asp Asn Ser
385                2390                2395                2400
Asp Leu Asn Ser Asn Leu Ser Gln Ser Thr Leu Asp Ile His
               2405                2410                2
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2441 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Ala Glu Asn Leu Leu Asp Gly Pro Pro Asn Pro Lys Arg Ala Lys
 1               5                  10                  15
Leu Ser Ser Pro Gly Phe Ser Ala Asn Asp Asn Thr Asp Phe Gly Ser
            20                  25                  30
Leu Phe Asp Leu Glu Asn Asp Leu Pro Asp Glu Leu Ile Pro Asn Gly
        35                  40                  45
Glu Leu Ser Leu Leu Asn Ser Gly Asn Leu Val Pro Asp Ala Ala Ser
    50                  55                  60
Lys His Lys Gln Leu Ser Glu Leu Leu Arg Gly Gly Ser Gly Ser Ser
65                  70                  75                  80
```

```
Ile Asn Pro Gly Ile Gly Asn Val Ser Ala Ser Ser Pro Val Gln Gln
                85                  90                  95
Gly Leu Gly Gly Gln Ala Gln Gly Gln Pro Asn Ser Thr Asn Met Ala
            100                 105                 110
Ser Leu Gly Ala Met Gly Lys Ser Pro Leu Asn Gln Gly Asp Ser Ser
        115                 120                 125
Thr Pro Asn Leu Pro Lys Gln Ala Ala Ser Thr Ser Gly Pro Thr Pro
    130                 135                 140
Pro Ala Ser Gln Ala Leu Asn Pro Gln Ala Gln Lys Gln Val Gly Leu
145                 150                 155                 160
Val Thr Ser Ser Pro Ala Thr Ser Gln Thr Gly Pro Gly Ile Cys Met
                165                 170                 175
Asn Ala Asn Phe Asn Gln Thr His Pro Gly Leu Leu Asn Ser Asn Ser
            180                 185                 190
Gly His Ser Leu Met Asn Gln Ala Gln Gln Gly Gln Ala Gln Val Met
        195                 200                 205
Asn Gly Ser Leu Gly Ala Ala Gly Arg Gly Arg Gly Ala Gly Met Pro
    210                 215                 220
Tyr Pro Ala Pro Ala Met Gln Gly Ala Thr Ser Ser Val Leu Ala Glu
225                 230                 235                 240
Thr Leu Thr Gln Val Ser Pro Gln Met Ala Gly His Ala Gly Leu Asn
                245                 250                 255
Thr Ala Gln Ala Gly Gly Met Thr Lys Met Gly Met Thr Gly Thr Thr
            260                 265                 270
Ser Pro Phe Gly Gln Pro Phe Ser Gln Thr Gly Gly Gln Gln Met Gly
        275                 280                 285
Ala Thr Gly Val Asn Pro Gln Leu Ala Ser Lys Gln Ser Met Val Asn
    290                 295                 300
Ser Leu Pro Ala Phe Pro Thr Asp Ile Lys Asn Thr Ser Val Thr Thr
305                 310                 315                 320
Val Pro Asn Met Ser Gln Leu Gln Thr Ser Val Gly Ile Val Pro Thr
                325                 330                 335
Gln Ala Ile Ala Thr Gly Pro Thr Ala Asp Pro Glu Lys Arg Lys Leu
            340                 345                 350
Ile Gln Gln Gln Leu Val Leu Leu Leu His Ala His Lys Cys Gln Arg
        355                 360                 365
Arg Glu Gln Ala Asn Gly Glu Val Arg Ala Cys Ser Leu Pro His Cys
    370                 375                 380
Arg Thr Met Lys Asn Val Leu Asn His Met Thr His Cys Gln Ala Pro
385                 390                 395                 400
Lys Ala Cys Gln Val Ala His Cys Ala Ser Ser Arg Gln Ile Ile Ser
                405                 410                 415
His Trp Lys Asn Cys Thr Arg His Asp Cys Pro Val Cys Leu Pro Leu
            420                 425                 430
Lys Asn Ala Ser Asp Lys Arg Asn Gln Gln Thr Ile Leu Gly Ser Pro
        435                 440                 445
Ala Ser Gly Ile Gln Asn Thr Ile Gly Ser Val Gly Ala Gly Gln Gln
    450                 455                 460
Asn Ala Thr Ser Leu Ser Asn Pro Asn Pro Ile Asp Pro Ser Ser Met
465                 470                 475                 480
Gln Arg Ala Tyr Ala Ala Leu Gly Leu Pro Tyr Met Asn Gln Pro Gln
                485                 490                 495
Thr Gln Leu Gln Pro Gln Val Pro Gly Gln Gln Pro Ala Gln Pro Pro
            500                 505                 510
Ala His Gln Gln Met Arg Thr Leu Asn Ala Leu Gly Asn Asn Pro Met
        515                 520                 525
Ser Val Pro Ala Gly Gly Ile Thr Thr Asp Gln Gln Pro Pro Asn Leu
    530                 535                 540
Ile Ser Glu Ser Ala Leu Pro Thr Ser Leu Gly Ala Thr Asn Pro Leu
545                 550                 555                 560
Met Asn Asp Gly Ser Asn Ser Gly Asn Ile Gly Ser Leu Ser Thr Ile
                565                 570                 575
```

```
Pro Thr Ala Ala Pro Pro Ser Ser Thr Gly Val Arg Lys Gly Trp His
            580                 585                 590
Glu His Val Thr Gln Asp Leu Arg Ser His Leu Val His Lys Leu Val
        595                 600                 605
Gln Ala Ile Phe Pro Thr Pro Asp Pro Ala Ala Leu Lys Asp Arg Arg
    610                 615                 620
Met Glu Asn Leu Val Ala Tyr Ala Lys Lys Val Glu Gly Asp Met Tyr
625                 630                 635                 640
Glu Ser Ala Asn Ser Arg Asp Glu Tyr Tyr His Leu Leu Ala Glu Lys
                645                 650                 655
Ile Tyr Lys Ile Gln Lys Glu Leu Glu Glu Lys Arg Arg Thr Arg Leu
            660                 665                 670
His Lys Gln Gly Ile Leu Gly Asn Gln Pro Ala Leu Pro Ala Ser Gly
        675                 680                 685
Ala Gln Pro Pro Val Ile Pro Pro Ala Gln Ser Val Arg Pro Pro Asn
    690                 695                 700
Gly Pro Leu Pro Leu Pro Val Asn Arg Met Gln Val Ser Gln Gly Met
705                 710                 715                 720
Asn Ser Phe Asn Pro Met Ser Leu Gly Asn Val Gln Leu Pro Gln Ala
                725                 730                 735
Pro Met Gly Pro Arg Ala Ala Ser Pro Met Asn His Ser Val Gln Met
            740                 745                 750
Asn Ser Met Ala Ser Val Pro Gly Met Ala Ile Ser Pro Ser Arg Met
        755                 760                 765
Pro Gln Pro Pro Asn Met Met Gly Thr His Ala Asn Asn Ile Met Ala
    770                 775                 780
Gln Ala Pro Thr Gln Asn Gln Phe Leu Pro Gln Asn Gln Phe Pro Ser
785                 790                 795                 800
Ser Ser Gly Ala Met Ser Val Asn Ser Val Gly Met Gly Gln Pro Ala
                805                 810                 815
Ala Gln Ala Gly Val Ser Gln Gly Gln Glu Pro Gly Ala Ala Leu Pro
            820                 825                 830
Asn Pro Leu Asn Met Leu Ala Pro Gln Ala Ser Gln Leu Pro Cys Pro
        835                 840                 845
Pro Val Thr Gln Ser Pro Leu His Pro Thr Pro Pro Pro Ala Ser Thr
    850                 855                 860
Ala Ala Gly Met Pro Ser Leu Gln His Pro Thr Ala Pro Gly Met Thr
865                 870                 875                 880
Pro Pro Gln Pro Ala Ala Pro Thr Gln Pro Ser Thr Pro Val Ser Ser
                885                 890                 895
Gly Gln Thr Pro Thr Pro Thr Pro Gly Ser Val Pro Ser Ala Ala Gln
            900                 905                 910
Thr Gln Ser Thr Pro Thr Val Gln Ala Ala Ala Gln Ala Gln Val Thr
        915                 920                 925
Pro Gln Pro Gln Thr Pro Val Gln Pro Pro Ser Val Ala Thr Pro Gln
    930                 935                 940
Ser Ser Gln Gln Gln Pro Thr Pro Val His Thr Gln Pro Pro Gly Thr
945                 950                 955                 960
Pro Leu Ser Gln Ala Ala Ala Ser Ile Asp Asn Arg Val Pro Thr Pro
                965                 970                 975
Ser Thr Val Thr Ser Ala Glu Thr Ser Ser Gln Gln Pro Gly Pro Asp
            980                 985                 990
Val Pro Met Leu Glu Met Lys Thr Glu Val Gln Thr Asp Asp Ala Glu
        995                1000                1005
Pro Glu Pro Thr Glu Ser Lys Gly Glu Pro Arg Ser Glu Met Met Glu
   1010                1015                1020
Glu Asp Leu Gln Gly Ser Ser Gln Val Lys Glu Glu Thr Asp Thr Thr
025                1030                1035                1040
Glu Gln Lys Ser Glu Pro Met Glu Val Glu Glu Lys Lys Pro Glu Val
               1045                1050                1055
Lys Val Glu Ala Lys Glu Glu Glu Glu Asn Ser Ser Asn Asp Thr Ala
           1060                1065                1070
```

```
Ser Gln Ser Thr Ser Pro Ser Gln Pro Arg Lys Lys Ile Phe Lys Pro
      1075                1080                1085
Glu Glu Leu Arg Gln Ala Leu Met Pro Thr Leu Glu Ala Leu Tyr Arg
   1090                1095                1100
Gln Asp Pro Glu Ser Leu Pro Phe Arg Gln Pro Val Asp Pro Gln Leu
105                1110                1115                1120
Leu Gly Ile Pro Asp Tyr Phe Asp Ile Val Lys Asn Pro Met Asp Leu
                1125                1130                1135
Ser Thr Ile Lys Arg Lys Leu Asp Thr Gly Gln Tyr Gln Glu Pro Trp
            1140                1145                1150
Gln Tyr Val Asp Asp Val Arg Leu Met Phe Asn Asn Ala Trp Leu Tyr
      1155                1160                1165
Asn Arg Lys Thr Ser Arg Val Tyr Lys Phe Cys Ser Lys Leu Ala Glu
   1170                1175                1180
Val Phe Glu Gln Glu Ile Asp Pro Val Met Gln Ser Leu Gly Tyr Cys
185                1190                1195                1200
Cys Gly Arg Lys Tyr Glu Phe Ser Pro Gln Thr Leu Cys Cys Tyr Gly
                1205                1210                1215
Lys Gln Leu Cys Thr Ile Pro Arg Asp Ala Ala Tyr Tyr Ser Tyr Gln
            1220                1225                1230
Asn Arg Tyr His Phe Cys Gly Lys Cys Phe Thr Glu Ile Gln Gly Glu
      1235                1240                1245
Asn Val Thr Leu Gly Asp Asp Pro Ser Gln Pro Gln Thr Thr Ile Ser
   1250                1255                1260
Lys Asp Gln Phe Glu Lys Lys Lys Asn Asp Thr Leu Asp Pro Glu Pro
265                1270                1275                1280
Phe Val Asp Cys Lys Glu Cys Gly Arg Lys Met His Gln Ile Cys Val
                1285                1290                1295
Leu His Tyr Asp Ile Ile Trp Pro Ser Gly Phe Val Cys Asp Asn Cys
            1300                1305                1310
Leu Lys Lys Thr Gly Arg Pro Arg Lys Glu Asn Lys Phe Ser Ala Lys
      1315                1320                1325
Arg Leu Gln Thr Thr Arg Leu Gly Asn His Leu Glu Asp Arg Val Asn
   1330                1335                1340
Lys Phe Leu Arg Arg Gln Asn His Pro Glu Ala Gly Glu Val Phe Val
345                1350                1355                1360
Arg Val Val Ala Ser Ser Asp Lys Thr Val Glu Val Lys Pro Gly Met
                1365                1370                1375
Lys Ser Arg Phe Val Asp Ser Gly Glu Met Ser Glu Ser Phe Pro Tyr
            1380                1385                1390
Arg Thr Lys Ala Leu Phe Ala Phe Glu Glu Ile Asp Gly Val Asp Val
      1395                1400                1405
Cys Phe Phe Gly Met His Val Gln Asp Thr Ala Leu Ile Ala Pro His
   1410                1415                1420
Gln Ile Gln Gly Cys Val Tyr Ile Ser Tyr Leu Asp Ser Ile His Phe
425                1430                1435                1440
Phe Arg Pro Arg Cys Leu Arg Thr Ala Val Tyr His Glu Ile Leu Ile
                1445                1450                1455
Gly Tyr Leu Glu Tyr Val Lys Lys Leu Val Tyr Val Thr Ala His Ile
            1460                1465                1470
Trp Ala Cys Pro Pro Ser Glu Gly Asp Asp Tyr Ile Phe His Cys His
      1475                1480                1485
Pro Pro Asp Gln Lys Ile Pro Lys Pro Lys Arg Leu Gln Glu Trp Tyr
   1490                1495                1500
Lys Lys Met Leu Asp Lys Ala Phe Ala Glu Arg Ile Ile Asn Asp Tyr
505                1510                1515                1520
Lys Asp Ile Phe Lys Gln Ala Asn Glu Asp Arg Leu Thr Ser Ala Lys
                1525                1530                1535
Glu Leu Pro Tyr Phe Glu Gly Asp Phe Trp Pro Asn Val Leu Glu Glu
            1540                1545                1550
Ser Ile Lys Glu Leu Glu Gln Glu Glu Glu Glu Arg Lys Lys Glu Glu
      1555                1560                1565
```

```
Ser Thr Ala Ala Ser Glu Thr Pro Glu Gly Ser Gln Gly Asp Ser Lys
   1570                1575                1580
Asn Ala Lys Lys Lys Asn Asn Lys Lys Thr Asn Lys Asn Lys Ser Ser
585                 1590                1595                1600
Ile Ser Arg Ala Asn Lys Lys Lys Pro Ser Met Pro Asn Val Ser Asn
                1605                1610                1615
Asp Leu Ser Gln Lys Leu Tyr Ala Thr Met Glu Lys His Lys Glu Val
            1620                1625                1630
Phe Phe Val Ile His Leu His Ala Gly Pro Val Ile Ser Thr Gln Pro
        1635                1640                1645
Pro Ile Val Asp Pro Asp Pro Leu Leu Ser Cys Asp Leu Met Asp Gly
   1650                1655                1660
Arg Asp Ala Phe Leu Thr Leu Ala Arg Asp Lys His Trp Glu Phe Ser
665                 1670                1675                1680
Ser Leu Arg Arg Ser Lys Trp Ser Thr Leu Cys Met Leu Val Glu Leu
                1685                1690                1695
His Thr Gln Gly Gln Asp Arg Phe Val Tyr Thr Cys Asn Glu Cys Lys
            1700                1705                1710
His His Val Glu Thr Arg Trp His Cys Thr Val Cys Glu Asp Tyr Asp
        1715                1720                1725
Leu Cys Ile Asn Cys Tyr Asn Thr Lys Ser His Thr His Lys Met Val
   1730                1735                1740
Lys Trp Gly Leu Gly Leu Asp Asp Glu Gly Ser Ser Gln Gly Glu Pro
745                 1750                1755                1760
Gln Ser Lys Ser Pro Gln Glu Ser Arg Arg Leu Ser Ile Gln Arg Cys
                1765                1770                1775
Ile Gln Ser Leu Val His Ala Cys Gln Cys Arg Asn Ala Asn Cys Ser
            1780                1785                1790
Leu Pro Ser Cys Gln Lys Met Lys Arg Val Val Gln His Thr Lys Gly
        1795                1800                1805
Cys Lys Arg Lys Thr Asn Gly Gly Cys Pro Val Cys Lys Gln Leu Ile
   1810                1815                1820
Ala Leu Cys Cys Tyr His Ala Lys His Cys Gln Glu Asn Lys Cys Pro
825                 1830                1835                1840
Val Pro Phe Cys Leu Asn Ile Lys His Asn Val Arg Gln Gln Gln Ile
                1845                1850                1855
Gln His Cys Leu Gln Gln Ala Gln Leu Met Arg Arg Arg Met Ala Thr
            1860                1865                1870
Met Asn Thr Arg Asn Val Pro Gln Gln Ser Leu Pro Ser Pro Thr Ser
        1875                1880                1885
Ala Pro Pro Gly Thr Pro Thr Gln Gln Pro Ser Thr Pro Gln Thr Pro
   1890                1895                1900
Gln Pro Pro Ala Gln Pro Gln Pro Ser Pro Val Asn Met Ser Pro Ala
905                 1910                1915                1920
Gly Phe Pro Asn Val Ala Arg Thr Gln Pro Pro Thr Ile Val Ser Ala
                1925                1930                1935
Gly Lys Pro Thr Asn Gln Val Pro Ala Pro Pro Pro Pro Ala Gln Pro
            1940                1945                1950
Pro Pro Ala Ala Val Glu Ala Ala Arg Gln Ile Glu Arg Glu Ala Gln
        1955                1960                1965
Gln Gln Gln His Leu Tyr Arg Ala Asn Ile Asn Asn Gly Met Pro Pro
   1970                1975                1980
Gly Arg Asp Gly Met Gly Thr Pro Gly Ser Gln Met Thr Pro Val Gly
985                 1990                1995                2000
Leu Asn Val Pro Arg Pro Asn Gln Val Ser Gly Pro Val Met Ser Ser
                2005                2010                2015
Met Pro Pro Gly Gln Trp Gln Gln Ala Pro Ile Pro Gln Gln Gln Pro
            2020                2025                2030
Met Pro Gly Met Pro Arg Pro Val Met Ser Met Gln Ala Gln Ala Ala
        2035                2040                2045
Val Ala Gly Pro Arg Met Pro Asn Val Gln Pro Asn Arg Ser Ile Ser
   2050                2055                2060
```

```
Pro Ser Ala Leu Gln Asp Leu Leu Arg Thr Leu Lys Ser Pro Ser Ser
065                 2070                2075                2080
Pro Gln Gln Gln Gln Gln Val Leu Asn Ile Leu Lys Ser Asn Pro Gln
                2085                2090                2095
Leu Met Ala Ala Phe Ile Lys Gln Arg Thr Ala Lys Tyr Val Ala Asn
            2100                2105                2110
Gln Pro Gly Met Gln Pro Gln Pro Gly Leu Gln Ser Gln Pro Gly Met
        2115                2120                2125
Gln Pro Gln Pro Gly Met His Gln Gln Pro Ser Leu Gln Asn Leu Asn
    2130                2135                2140
Ala Met Gln Ala Gly Val Pro Arg Pro Gly Val Pro Pro Pro Gln Pro
145                 2150                2155                2160
Ala Met Gly Gly Leu Asn Pro Gln Gly Gln Ala Leu Asn Ile Met Asn
                2165                2170                2175
Pro Gly His Asn Pro Asn Met Thr Asn Met Asn Pro Gln Tyr Arg Glu
            2180                2185                2190
Met Val Arg Arg Gln Leu Leu Gln His Gln Gln Gln Gln Gln Gln Gln
        2195                2200                2205
Gln Gln Gln Gln Gln Gln Gln Gln Asn Ser Ala Ser Leu Ala Gly Gly
    2210                2215                2220
Met Ala Gly His Ser Gln Phe Gln Gln Pro Gln Gly Pro Gly Gly Tyr
225                 2230                2235                2240
Ala Pro Ala Met Gln Gln Gln Arg Met Gln Gln His Leu Pro Ile Gln
                2245                2250                2255
Gly Ser Ser Met Gly Gln Met Ala Ala Pro Met Gly Gln Leu Gly Gln
            2260                2265                2270
Met Gly Gln Pro Gly Leu Gly Ala Asp Ser Thr Pro Asn Ile Gln Gln
        2275                2280                2285
Ala Leu Gln Gln Arg Ile Leu Gln Gln Gln Gln Met Lys Gln Gln Ile
    2290                2295                2300
Gly Ser Pro Gly Gln Pro Asn Pro Met Ser Pro Gln Gln His Met Leu
305                 2310                2315                2320
Ser Gly Gln Pro Gln Ala Ser His Leu Pro Gly Gln Gln Ile Ala Thr
                2325                2330                2335
Ser Leu Ser Asn Gln Val Arg Ser Pro Ala Pro Val Gln Ser Pro Arg
            2340                2345                2350
Pro Gln Ser Gln Pro Pro His Ser Ser Pro Ser Pro Arg Ile Gln Pro
        2355                2360                2365
Gln Pro Ser Pro His His Val Ser Pro Gln Thr Gly Thr Pro His Pro
    2370                2375                2380
Gly Leu Ala Val Thr Met Ala Ser Ser Met Asp Gln Gly His Leu Gly
385                 2390                2395                2400
Asn Pro Glu Gln Ser Ala Met Leu Pro Gln Leu Asn Thr Pro Asn Arg
                2405                2410                2415
Ser Ala Leu Ser Ser Glu Leu Ser Leu Val Gly Asp Thr Thr Gly Asp
            2420                2425                2430
Thr Leu Glu Lys Phe Val Glu Gly Leu
        2435                2440
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 813 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: None

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Ala Glu Ala Gly Gly Ala Gly Ser Pro Ala Leu Pro Pro Ala Pro
 1               5                  10                  15
```

```
Pro His Gly Ser Pro Arg Thr Leu Ala Thr Ala Ala Gly Ser Ser Ala
            20                  25                  30
Ser Cys Gly Pro Ala Thr Pro Val Ala Ala Ala Gly Thr Ala Glu Gly
        35                  40                  45
Pro Gly Gly Gly Gly Ser Ala Arg Ile Ala Val Lys Lys Ala Gln Leu
    50                  55                  60
Arg Ser Ala Pro Arg Ala Lys Lys Leu Glu Lys Leu Gly Val Tyr Ser
65                  70                  75                  80
Ala Cys Lys Ala Glu Glu Ser Cys Lys Cys Asn Gly Trp Lys Asn Pro
                85                  90                  95
Asn Pro Ser Pro Thr Pro Pro Arg Gly Asp Leu Gln Gln Ile Ile Val
            100                 105                 110
Ser Leu Thr Glu Ser Cys Arg Ser Cys Ser His Ala Leu Ala Ala His
        115                 120                 125
Val Ser His Leu Glu Asn Val Ser Glu Glu Glu Met Asp Arg Leu Leu
    130                 135                 140
Gly Ile Val Leu Asp Val Glu Tyr Leu Phe Thr Cys Val His Lys Glu
145                 150                 155                 160
Glu Asp Ala Asp Thr Lys Gln Val Tyr Phe Tyr Leu Phe Lys Leu Leu
                165                 170                 175
Arg Lys Ser Ile Leu Gln Arg Gly Lys Pro Val Val Glu Gly Ser Leu
            180                 185                 190
Glu Lys Lys Pro Pro Phe Glu Lys Pro Ser Ile Glu Gln Gly Val Asn
        195                 200                 205
Asn Phe Val Gln Tyr Lys Phe Ser His Leu Pro Ser Lys Glu Arg Gln
    210                 215                 220
Thr Thr Ile Glu Leu Ala Lys Met Phe Leu Asn Arg Ile Asn Tyr Trp
225                 230                 235                 240
His Leu Glu Ala Pro Ser Gln Arg Arg Leu Arg Ser Pro Asn Asp Asp
                245                 250                 255
Ile Ser Gly Tyr Lys Glu Asn Tyr Thr Arg Trp Leu Cys Tyr Cys Asn
            260                 265                 270
Val Pro Gln Phe Cys Asp Ser Leu Pro Arg Tyr Glu Thr Thr Lys Val
        275                 280                 285
Phe Gly Arg Thr Leu Leu Arg Ser Val Phe Thr Ile Met Arg Arg Gln
    290                 295                 300
Leu Leu Glu Gln Ala Arg Gln Lys Lys Asp Lys Leu Pro Leu Glu Lys
305                 310                 315                 320
Arg Thr Leu Ile Leu Thr His Phe Pro Lys Phe Leu Ser Met Leu Glu
                325                 330                 335
Glu Glu Val Tyr Ser Gln Asn Ser Pro Ile Trp Asp Gln Asp Phe Leu
            340                 345                 350
Ser Ala Ser Ser Arg Thr Ser Pro Leu Gly Ile Gln Thr Val Ile Ser
        355                 360                 365
Pro Pro Val Thr Gly Thr Ala Leu Phe Ser Ser Asn Ser Thr Ser His
    370                 375                 380
Glu Gln Ile Asn Gly Gly Arg Thr Ser Pro Gly Cys Arg Gly Ser Ser
385                 390                 395                 400
Gly Leu Glu Ala Asn Pro Gly Glu Lys Arg Lys Met Asn Asn Ser His
                405                 410                 415
Ala Pro Glu Glu Ala Lys Arg Ser Arg Val Met Gly Asp Ile Pro Val
            420                 425                 430
Glu Leu Ile Asn Glu Val Met Ser Thr Ile Thr Asp Pro Ala Gly Met
        435                 440                 445
Leu Gly Pro Glu Thr Asn Phe Leu Ser Ala His Ser Ala Arg Asp Glu
    450                 455                 460
Ala Ala Arg Leu Glu Glu Arg Arg Gly Val Ile Glu Phe His Val Val
465                 470                 475                 480
Gly Asn Ser Leu Asn Gln Lys Pro Asn Lys Lys Ile Leu Met Trp Leu
                485                 490                 495
Val Gly Leu Gln Asn Val Phe Ser His Gln Leu Pro Arg Met Pro Lys
            500                 505                 510
```

```
Glu Tyr Ile Thr Arg Leu Val Phe Asp Pro Lys His Lys Thr Leu Ala
        515                 520                 525
Leu Ile Lys Asp Gly Arg Val Ile Gly Gly Ile Cys Phe Arg Met Phe
    530                 535                 540
Pro Ser Gln Gly Phe Thr Glu Ile Val Phe Cys Ala Val Thr Ser Asn
545                 550                 555                 560
Glu Gln Val Lys Gly Tyr Gly Thr His Leu Met Asn His Leu Lys Glu
                565                 570                 575
Tyr His Ile Lys His Glu Ile Leu Asn Phe Leu Thr Tyr Ala Asp Glu
            580                 585                 590
Tyr Ala Ile Gly Tyr Phe Lys Lys Gln Gly Phe Ser Lys Glu Ile Lys
        595                 600                 605
Ile Pro Lys Thr Lys Tyr Val Gly Tyr Ile Lys Asp Tyr Glu Gly Ala
    610                 615                 620
Thr Leu Met Gly Cys Glu Leu Asn Pro Gln Ile Pro Tyr Thr Glu Phe
625                 630                 635                 640
Ser Val Ile Ile Lys Lys Gln Lys Glu Ile Ile Lys Lys Leu Ile Glu
                645                 650                 655
Arg Lys Gln Ala Gln Ile Arg Lys Val Tyr Pro Gly Leu Ser Cys Phe
            660                 665                 670
Lys Asp Gly Val Arg Gln Ile Pro Ile Glu Ser Ile Pro Gly Ile Arg
        675                 680                 685
Glu Thr Gly Trp Lys Pro Ser Gly Lys Glu Lys Ser Lys Glu Pro Lys
    690                 695                 700
Asp Pro Glu His Val Tyr Ser Thr Leu Lys Asn Ile Leu Gln Gln Val
705                 710                 715                 720
Lys Asn His Pro Asn Ala Trp Pro Phe Met Glu Pro Val Lys Arg Thr
                725                 730                 735
Glu Ala Pro Gly Tyr Tyr Glu Val Ile Arg Phe Pro Met Asp Leu Lys
            740                 745                 750
Thr Met Ser Glu Arg Leu Arg Asn Arg Tyr Tyr Val Ser Lys Lys Leu
        755                 760                 765
Phe Met Ala Asp Leu Gln Arg Val Phe Thr Asn Cys Lys Glu Tyr Asn
    770                 775                 780
Pro Pro Glu Ser Glu Tyr Tyr Lys Cys Ala Ser Ile Leu Glu Lys Phe
785                 790                 795                 800
Phe Phe Ser Lys Ile Lys Glu Ala Gly Leu Ile Asp Lys
                805                 810
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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His Thr Lys Gly Cys Lys Arg Lys Thr Asn Gly Gly Cys Pro Val Cys
 1               5                  10                  15
Lys Gln Leu Ile Ala Leu Cys Cys Tyr His Ala Lys His Cys Gln Glu
            20                  25                  30
Asn Lys Cys Pro Val Pro Phe Cys Leu Asn Ile Lys His Asn Val Arg
        35                  40                  45
Gln Gln
    50
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2204 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACCCACTCCC CCCAGAGCCG ACCTGCAGCA AATAATTGTC AGTCTAACAG AATCCTGTCG 60
GAGTTGTAGC CATGCCCTAG CTGCTCATGT TTCCCACCTG GAGAATGTGT CAGAGGAAGA 120
AATGAACAGA CTCCTGGGAA TAGTATTGGA TGTGGAATAT CTCTTTACCT GTGTCCACAA 180
GGAAGAAGAT GCAGATACCA AACAAGTTTA TTTCTATCTA TTTAAGCTCT TGAGAAAGTC 240
TATTTTACAA AGAGGAAAAC CTGTGGTTGG AAGGCTCTTT GGAAAAGAAA CCCCCATTTG 300
AAAAACCTAG CATTGAACAG GGTGTGAATA ACTTTGTGCA GTACAAATTT AGTCACCTGC 360
CAGCAAAAAG AAAGGCAAAC CAATAGTTGA GTTGGCAAAA ATGTTCCTAA ACCGCATCAC 420
CTATTGGCAT CTGGAGGCAC CATCTCAACG AGACTGCGAT CTCCAATGAT GATATTCTGG 480
ATACAAAGAG AACTACACAA GGTGGCTGTG TTACTGCAAC GTGCCACAGT TCTGCGACAG 540
TCTACCTCGG TACGAAACCA CACAGGTGTT TGGGAGAACA TCGTTCGCTC GGTCTTCACT 600
GTTATGAGGC GACAACTCCT GGAACAAGCA AGACAGGAAA AAGATAAACT GCCTCTTGAA 660
AAACGAACTC TAATCCTCAC TCATTTCCCA AAATTTCTGT CCATGCTAGA AGAAGAAGTA 720
TATAGTCAAA ACTCTCCCAT CTGGGATCAC CATTTTCTCT CAGCCTCTTC CAGAACCAGC 780
CAGCTAGGCA TCCAAACAGT TATCAATCAC CTCCTGTGGC TGGGACAATT TCATACAATT 840
CAACCTCATC TTCCCTTGAG CAGCCAAACG CAGGGAGCAG CAGTCCTGCC TGCAAAGCCT 900
CTTCTGGACT TGAGGCAAAC CCAGGAGAAA AGAGGAAAAT GACTGATTCT CATGTTCTGG 960
AGGAGGCCAA GAAACCCCGA GTTATGGGGG ATATTCCGAT GGAATTAATC AACGAGGTTA 1020
TGTCTACCAT CACGGACCCT GCAGCAATGC TTGGACCAGA GACCAATTTT CTGTCAGCAC 1080
ACTCGGCCAG GGATGAGGCG GCAAGGTTGG AAGAGCGCAG GGGTGTAATT GAATTTCACG 1140
TGGTTGGCAA TTCCCTCAAC CAGAAACCAA ACAAGAAGAT CCTGATGTGG CTGGTTGGCC 1200
TACAGAACGT TTTCTCCCAC CAGCTGCCCC GAATGCCAAA AGAATACATC ACACGGCTCG 1260
TCTTTGACCC GAAACACAAA ACCCTTGCTT TAATTAAAGA TGGCCGTGTT ATTGGTGGTA 1320
TCTGTTTCCG TATGTTCCCA TCTCAAGGAT TCACAGAGAT TGTCTTCTGT GCTGTAACCT 1380
CAAATGAGCA AGTCAAGGGC TATGGAACAC ACCTGATGAA TCATTTGAAA GAATATCACA 1440
TAAAGCATGA CATCCTGAAC TTCCTCACAT ATGCAGATGA ATATGCAATT GGATACTTTA 1500
AGAAACAGGG TTTCTCCAAA GAAATTAAAA TACCTAAAAC CAAATATGTT GGCTATATCA 1560
AGGATTATGA AGGAGCCACT TTAATGGGAT GTGAGCTAAA TCCACGGATC CCGTACACAG 1620
AATTTTCTGT CATCATTAAA AAGCAGAAGG AGATAATTAA AAAACTGATT GAAAGAAAAC 1680
AGGCACAAAT TCGAAAAGTT TACCCTGGAC TTTCATGTTT TAAAGATGGA GTTCGACAGA 1740
TTCCTATAGA AAGCATTCCT GGAATTAGAG AGACAGGCTG GAAACCGAGT GGAAAAGAGA 1800
AAAGTAAAGA GCCCAGAGAC CCTGACCAGC TTTACAGCAC GCTCAAGAGC ATCCTCCAGC 1860
AGGTGAAGAG CCATCAAAGC GCTTGGCCCT TCATGGAACC TGTGAAGAGA ACAGAAGCTC 1920
CAGGATATTA TGAAGTTATA AGGTCCCCCA TGGATCTCAA AACCATGAGT GAACGCCTCA 1980
AGAATAGGTA CTACGTGTCT AAGAAATTAT TCATGGCAGA CTTACAGCGA GTCTTTACCA 2040
ATTGCAAAGA GTACAACGCC CCTGAGAGTG AATACTACAA ATGTGCCAAT ATCCTGGAGA 2100
AATTCTTCTT CAGTAAAATT AAGGAAGCTG GATTAATTGA CAAGTGATTT TTTTTCCCCC 2160
TCTGCTTCTT AGAAACTCAC CAAGCAGTGT GCCTAAAGCA AGGT 2204

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2093 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTCCGGC GAAACCACTC ATGTCTTTGG GCGAAGCCTT CTCCGGTCCA TTTTCACCGT 60
TACCCGCCGG CAGCTGCTGG AAAAGTTCCG AGTGGAGAAG GACAAATTGG TGCCCGAGAA 120
GAGGACCCTC ATCCTCACTC ACTTCCCCAA GTAAGGCTCC TTCTGGCCTA CCAGGATTTG 180
GCCCCAAGTT CACATCCTCC CTGTTGTCCC CTTTTTTCCA GGAAGGCTTC CTGGATTGGT 240
CCCTCCTCTC CCTCCATGGG CCTTTTGGGA TCTGGGCGTC TACCTGGCAG ACTTGCCCAT 300
GGCCCAGAAG CAACTTGCTA GTACTAGTCT GGGGATGGCA GATTCCTGTC CATGCTGGAG 360
GAGGAGATCT ATGGGGCAAA CTCTCCAATC TGGGAGTCAG GCTTCACCAT GCCACCCTCA 420

GAGGGGACAC AGCTGGTTCC CCGGCCAGCT TCAGTCAGTG CAGCGGTTGT TCCCAGCACC 480
CCCATCTTCA GCCCCAGCAT GGGTGGGGGC AGCAACAGCT CCCTGAGTCT GGATTCTGCA 540
GGGGCCGAGC CTATGCCAGG CGAGAAGAGG ACGCTCCCAG AGAACCTGAC CCTGGAGGAT 600
GCCAAGCGGC TCCGTGTGAT GGGTGACATC CCCATGGAGC TGGTCAATGA GGTCATGCTG 660
ACCATCACTG ACCCTGCTGC CATGCTGGGG CCTGAGACGA GCCTGCTTTC GGCCAATGCG 720
GCCCGGGATG AGACAGCCCG CCTGGAGGAG CGCCGCGGCA TCATCGAGTT CCATGTCATC 780
GGCAACTCAC TGACGCCCAA GGCCAACCGG CGGGTGTTGC TGTGGCTCGT GGGGCTGCAG 840
AATGTCTTTT CCCACCAGCT GCCGCGCATG CCTAAGGAGT ATATCGCCCG CCTCGTCTTT 900
GACCCGAAGC ACAAGACTCT GGCCTTGATC AAGGATGGGC GGGTCATCGG TGGCATCTGC 960
TTCCGCATGT TTCCCACCCA GGGCTTCACG GAGATTGTCT TCTGTGCTGT CACCTCGAAT 1020
GAGCAGGTCA AGGGTTATGG GACCCACCTG ATGAACCACC TGAAGGAGTA TCACATCAAG 1080
CACAACATTC TCTACTTCCT CACCTACGCC GACGAGTACG CCATCGGCTA CTTCAAAAAG 1140
CAGGGTTTCT CCAAGGACAT CAAGGTGCCC AAGAGCCGCT ACCTGGGCTA CATCAAGGAC 1200
TACGAGGGAG CGACGCTGAT GGAGTGTGAG CTGAATCCCC GCATCCCCTA CACGGAGCTG 1260
TCCCACATCA TCAAGAAGCA GAAAGAGATC ATCAAGAAGC TGATTGAGCG CAAACAGGCC 1320
CAGATCCGCA AGGTCTACCC GGGGCTCAGC TGCTTCAAGG AGGGCGTGAG GCAGATCCCT 1380
GTGGAGAGCG TTCCTGGCAT TCGAGAGACA GGCTGGAAGC CATTGGGGAA GGAGAAGGGG 1440
AAGGAGCTGA AGGACCCCGA CCAGCTCTAC ACAACCCTCA AAAACCTGCT GGCCCAAATC 1500
AAGTCTCACC CCAGTGCCTG GCCCTTCATG GAGCCTGTGA AGAAGTCGGA GGCCCCTGAC 1560
TACTACGAGG TCATCCGCTT CCCCATTGAC CTGAAGACCA TGACTGAGCG GCTGCGAAGC 1620
CGCTACTACG TGACCCGGAA GCTCTTTGTG GCCGACCTGC AGCGGGTCAT CGCCAACTGT 1680
CGCGAGTACA ACCCCCCGGA CAGCGAGTAC TGCCGCTGTG CCAGCGCCCT GGAGAAGTTC 1740
TTCTACTTCA AGCTCAAGGA GGGAGGCCTC ATTGACAAGT AGGCCCATCT TTGGGCCGCA 1800
GCCCTGACCT GGAATGTCTC CACCTCGGAT TCTGATCTGA TCCTTAGGGG GTGCCCTGGC 1860
CCCACGGACC CGACTCAGCT TGAGACACTC CAGCCAAGGG TCCTCCGGAC CCGATCCTGC 1920
AGCTCTTTCT GGACCTTCAG GCACCCCCAA GCGTGCAGCT CTGTCCCAGC CTTCACTGTG 1980
TGTGAGAGGT CTCCTGGGTT GGGGCCCAGC CCCTCTAGAG TAGCTGGTGG CCAGGGATGA 2040
ACCTTGCCCA GCCGTGGTGG CCCCCAGGCC TGGTCCCCAA GAGCCCGGAA TTC 2093

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9046 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTTGTTTGT GTGCTAGGCT GGGGGGGAGA GAGGGCGAGA GAGAGCGGGC GAGAGTGGGC 60
AAGCAGGACG CCGGGCTGAG TGCTAACTGC GGGACGCAGA GAGTGCGGAG GGGAGTCGGG 120
TCGGAGAGAG GCGGCAGGGG CCAGAACAGT GGCAGGGGGC CCGGGGCGCA CGGGCTGAGG 180
CGACCCCCAG CCCCCTCCCG TCCGCACACA CCCCCACCGC GGTCCAGCAG CCGGGCCGGC 240
GTCGACGCTA GGGGGGACCA TTACATAACC CGCGCCCCGG CCGTCTTCTC CCGCCGCCGC 300
GGCGCCCGAA CTGAGCCCGG GGCGGGCGCT CCAGCACTGG CCGCCGGCGT GGGGCGTAGC 360
AGCGGCCGTA TTATTATTTC GCGGAAAGGA AGGCGAAGGA GGGGAGCGCC GGCGCGAGGA 420
GGGGCCGCCT GCGCCCGCCG CCGGAGCGGG GCCTCCTCGG TGGGCTCCGC GTCGGCGCGG 480
GCGTGCGGGC GGCGCTGCTC GGCCCGGCCC CCTCGGCCCT CTGGTCCGGC CAGCTCCGCT 540
CCCGGCGTCC TTGCCGCGCC TCCGCCGGCC GCCGCGCGAT GTGAGGCGGC GGCGCCAGCC 600
TGGCTCTCGG CTCGGGCGAG TTCTCTGCGG CCATTAGGGG CCGGTGCGGC GGCGGCGCGG 660
AGCGCGGCGG CAGGAGGAGG GTTCGGAGGG TGGGGGCGCA GGCCCGGGAG GGGGCACCGG 720
GAGGAGGTGA GTGTCTCTTG TCGCCTCCTC CTCTCCCCCC TTTTCGCCCC CGCCTCCTTG 780
TGGCGATGAG AAGGAGGAGG ACAGCGCCGA GGAGGAAGAG GTTGATGGCG GCGGCGGAGC 840
TCCGAGAGAC CTCGGCTGGG CAGGGGCCGG CCGTGGCGGG CCGGGGACTG CGCCTCTAGA 900
GCCGCGAGTT CTCGGGAATT CGCCGCAGCG GACCGGCCTC GGCGAATTTG TGCTCTTGTG 960
CCCTCCTCCG GGCTTGGGCC AGGCCGGCCC CTCGCACTTG CCCTTACCTT TTCTATCGAG 1020
TCCGCATCCC TCTCCAGCCA CTGCGACCCG GCGAAGAGAA AAAGGAACTT CCCCCACCCC 1080
CTCGGGTGCC GTCGGAGCCC CCCAGCCCAC CCCTGGGTGC GGCGCGGGGA CCCCGGGCCG 1140
AAGAAGAGAT TTCCTGAGGA TTCTGGTTTT CCTCGCTTGT ATCTCCGAAA GAATTAAAAA 1200
TGGCCGAGAA TGTGGTGGAA CCGGGGCCGC CTTCAGCCAA GCGGCCTAAA CTCTCATCTC 1260
CGGCCCTCTC GGCGTCCGCC AGCGATGGCA CAGATTTTGG CTCTCTATTT GACTTGGAGC 1320
ACGACTTACC AGATGAATTA ATCAACTCTA CAGAATTGGG ACTAACCAAT GGTGGTGATA 1380

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TTAATCAGCT TCAGACAAGT CTTGGCATGG TACAAGATGC AGCTTCTAAA CATAAACAGC   1440
TGTCAGAATT GCTGCGATCT GGTAGTTCCC CTAACCTCAA TATGGGAGTT GGTGGCCCAG   1500
GTCAAGTCAT GGCCAGCCAG GCCCAACAGA GCAGTCCTGG ATTAGGTTTG ATAAATAGCA   1560
TGGTCAAAAG CCCAATGACA CAGGCAGGCT TGACTTCTCC CAACATGGGG ATGGGCACTA   1620
GTGGACCAAA TCAGGGTCCT ACGCAGTCAA CAGGTATGAT GAACAGTCCA GTAAATCAGC   1680
CTGCCATGGG AATGAACACA GGGACGAATG CGGGCATGAA TCCTGGAATG TTGGCTGCAG   1740
GCAATGGACA AGGGATAATG CCTAATCAAG TCATGAACGG TTCAATTGGA GCAGGCCGAG   1800
GGCGACAGGA TATGCAGTAC CCAAACCCAG GCATGGGAAG TGCTGGCAAC TTACTGACTG   1860
AGCCTCTTCA GCAGGGCTCT CCCCAGATGG GAGGACAAAC AGGATTGAGA GGCCCCCAGC   1920
CTCTTAAGAT GGGAATGATG AACAACCCCA ATCCTTATGG TTCACCATAT ACTCAGAATC   1980
CTGGACAGCA GATTGGAGCC AGTGGCCTTG GTCTCCAGAT TCAGACAAAA ACTGTACTAT   2040
CAAATAACTT ATCTCCATTT GCTATGGACA AAAAGGCAGT TCCTGGTGGA GGAATGCCCA   2100
ACATGGGTCA ACAGCCAGCC CCGCAGGTCC AGCAGCCAGG TCTGGTGACT CCAGTTGCCC   2160
AAGGGATGGG TTCTGGAGCA CATACAGCTG ATCCAGAGAA GCGCAAGCTC ATCCAGCAGC   2220
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TGAGGCAGTG CAACCTTCCC CACTGTCGCA CAATGAAGAA TGTCCTAAAC CACATGACAC   2340
ACTGCCAGTC AGGCAAGTCT TGCCAAGTGG CACACTGTGC ATCTTCTCGA CAAATCATTT   2400
CACACTGGAA GAATTGTACA AGACATGATT GTCCTGTGTG TCTCCCCCTC AAAAATGCTG   2460
GTGATAAGAG AAATCAACAG CCAATTTTGA CTGGAGCACC CGTTGGACTT GGAAATCCTA   2520
GCTCTCTAGG GGTGGGTCAA CAGTCTGCCC CCAACCTAAG CACTGTTAGT CAGATTGATC   2580
CCAGCTCCAT AGAAAGAGCC TATGCAGCTC TTGGACTACC CTATCAAGTA AATCAGATGC   2640
CGACACAACC CCAGGTGCAA GCAAAGAACC AGCAGAATCA GCAGCCTGGG CAGTCTCCCC   2700
AAGGCATGCG GCCCATGAGC AACATGAGTG CTAGTCCTAT GGGAGTAAAT GGAGGTGTAG   2760
GAGTTCAAAC GCCGAGTCTT CTTTCTGACT CAATGTTGCA TTCAGCCATA AATTCTCAAA   2820
ACCCAATGAT GAGTGAAAAT GCCAGTGTGC CCTCCCTGGG TCCTATGCCA ACAGCAGCTC   2880
AACCATCCAC TACTGGAATT CGGAAACAGT GGCACGAAGA TATTACTCAG GATCTTCGAA   2940
ATCATCTTGT TCACAAACTC GTCCAAGCCA TATTTCCTAC GCCGGATCCT GCTGCTTTAA   3000
AAGACAGACG GATGGAAAAC CTAGTTGCAT ATGCTCGGAA AGTTGAAGGG GACATGTATG   3060
AATCTGCAAA CAATCGAGCG GAATACTACC ACCTTCTAGC TGAGAAAATC TATAAGATCC   3120
AGAAAGAACT AGAAGAAAAA CGAAGGACCA GACTACAGAA GCAGAACATG CTACCAAATG   3180
CTGCAGGCAT GGTTCCAGTT TCCATGAATC CAGGGCCTAA CATGGGACAG CCGCAACCAG   3240
GAATGACTTC TAATGGCCCT CTACCTGACC CAAGTATGAT CCGTGGCAGT GTGCCAAACC   3300
AGATGATGCC TCGAATAACT CCACAATCTG GTTTGAATCA ATTTGGCCAG ATGAGCATGG   3360
CCCAGCCCCC TATTGTACCC CGGCAAACCC CTCCTCTTCA GCACCATGGA CAGTTGGCTC   3420
AACCTGGAGC TCTCAACCCG CCTATGGGCT ATGGGCCTCG TATGCAACAG CCTTCCAACC   3480
AGGGCCAGTT CCTTCCTCAG ACTCAGTTCC CATCACAGGG AATGAATGTA ACAAATATCC   3540
CTTTGGCTCC GTCCAGCGGT CAAGCTCCAG TGTCTCAAGC ACAAATGTCT AGTTCTTCCT   3600
GCCCGGTGAA CTCTCCTATA ATGCCTCCAG GGTCTCAGGG GAGCCACATT CACTGTCCCC   3660
AGCTTCCTCA ACCAGCTCTT CATCAGAATT CACCCTCGCC TGTACCTAGT CGTACCCCCA   3720
CCCCTCACCA TACTCCCCCA AGCATAGGGG CTCAGCAGCC ACCAGCAACA ACAATTCCAG   3780
CCCCTGTTCC TACACCACCA GCCATGCCAC CTGGGCCACA GTCCCAGGCT CTACATCCCC   3840
CTCCAAGGCA GACACCTACA CCACCAACAA CACAACTTCC CCAACAAGTG CAGCCTTCAC   3900
TTCCTGCTGC ACCTTCTGCT GACCAGCCCC AGCAGCAGCC TCGCTCACAG CAGAGCACAG   3960
CAGCGTCTGT TCCTACCCCA AACGCACCGC TGCTTCCTCC GCAGCCTGCA ACTCCACTTT   4020
CCCAGCCAGC TGTAAGCATT GAAGGACAGG TATCAAATCC TCCATCTACT AGTAGCACAG   4080
AAGTGAATTC TCAGGCCATT GCTGAGAAGC AGCCTTCCCA GGAAGTGAAG ATGGAGGCCA   4140
AAATGGAAGT GGATCAACCA GAACCAGCAG ATACGCAGCC GGAGGATATT TCAGAGTCTA   4200
AAGTGGAAGA CTGTAAAATG GAATCTACCG AAACAGAAGA GAGAAGCACT GAGTTAAAAA   4260
CTGAAATAAA AGAGGAGGAA GACCAGCCAA GTACTTCAGC TACCCAGTCA TCTCCGGCTC   4320
CAGGACAGTC AAAGAAAAAG ATTTTCAAAC CAGAAGAACT ACGACAGGCA CTGATGCCAA   4380
CATTGGAGGC ACTTTACCGT CAGGATCCAG AATCCCTTCC CTTTCGTCAA CCTGTGGACC   4440
CTCAGCTTTT AGGAATCCCT GATTACTTTG ATATTGTGAA GAGCCCCATG GATCTTTCTA   4500
CCATTAAGAG GAAGTTAGAC ACTGGACAGT ATCAGGAGCC CTGGCAGTAT GTCGATGATA   4560
TTTGGCTTAT GTTCAATAAT GCCTGGTTAT ATAACCGGAA AACATCACGG GTATACAAAT   4620
ACTGCTCCAA GCTCTCTGAG GTCTTTGAAC AAGAAATTGA CCCAGTGATG CAAAGCCTTG   4680
GATACTGTTG TGGCAGAAAG TTGGAGTTCT CTCCACAGAC ACTGTGTTGC TACGGCAAAC   4740
AGTTGTGCAC AATACCTCGT GATGCCACTT ATTACAGTTA CCAGAACAGG TATCATTTCT   4800
GTGAGAAGTG TTTCAATGAG ATCCAAGGGG AGAGCGTTTC TTTGGGGGAT GACCCTTCCC   4860
AGCCTCAAAC TACAATAAAT AAAGAACAAT TTTCCAAGAG AAAAAATGAC ACACTGGATC   4920
CTGAACTGTT TGTTGAATGT ACAGAGTGCG GAAGAAAGAT GCATCAGATC TGTGTCCTTC   4980
ACCATGAGAT CATCTGGCCT GCTGGATTCG TCTGTGATGG CTGTTTAAAG AAAAGTGCAC   5040
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TTCTAGAGAA TCGTGTGAAT GACTTTCTGA GGCGACAGAA TCACCCTGAG TCAGGAGAGG   5160
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TCACTGTTAG AGTAGTTCAT GCTTCTGACA AAACCGTGGA AGTAAAACCA GGCATGAAAG 5220
CAAGGTTTGT GGACAGTGGA GAGATGGCAG AATCCTTTCC ATACCGAACC AAAGCCCTCT 5280
TTGCCTTTGA AGAAATTGAT GGTGTTGACC TGTGCTTCTT TGGCATGCAT GTTCAAGAGT 5340
ATGGCTCTGA CTGCCCTCCA CCCAACCAGA GGAGAGTATA CATATCTTAC CTCGATAGTG 5400
TTCATTTCTT CCGTCCTAAA TGCTTGAGGA CTGCAGTCTA TCATGAAATC CTAATTGGAT 5460
ATTTAGAATA TGTCAAGAAA TTAGGTTACA CAACAGGGCA TATTTGGGCA TGTCCACCAA 5520
GTGAGGGAGA TGATTATATC TTCCATTGCC ATCCTCCTGA CCAGAAGATA CCCAAGCCCA 5580
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TGAAGCGGGT TGTGCAGCAT ACCAAGGGTT GCAAACGGAA AACCAATGGC GGGTGCCCCA 6540
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CAGCAGGCCA GGTGACCCCT CCAACCCCTC CTCAGACTGC TCAGCCACCC CTTCCAGGGC 6960
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ATGGTCAACC TTTGAACATG GCTCCACAAC CAGGATTGGG CCAGGTAGGT ATCAGCCCAC 7320
TCAAACCAGG CACTGTGTCT CAACAAGCCT TACAAAACCT TTTGCGGACT CTCAGGTCTC 7380
CCAGCTCTCC CCTGCAGCAG CAACAGGTGC TTAGTATCCT TCACGCCAAC CCCCAGCTGT 7440
TGGCTGCATT CATCAAGCAG CGGGCTGCCA AGTATGCCAA CTCTAATCCA CAACCCATCC 7500
CTGGGCAGCC TGGCATGCCC CAGGGGCAGC CAGGGCTACA GCCACCTACC ATGCCAGGTC 7560
AGCAGGGGGT CCACTCCAAT CCAGCCATGC AGAACATGAA TCCAATGCAG GCGGGCGTTC 7620
AGAGGGCTGG CCTGCCCCAG CAGCAACCAC AGCAGCAACT CCAGCCACCC ATGGGAGGGA 7680
TGAGCCCCCA GGCTCAGCAG ATGAACATGA ACCACAACAC CATGCCTTCA CAATTCCGAG 7740
ACATCTTGAG ACGACAGCAA ATGATGCAAC AGCAGCAGCA ACAGGGAGCA GGGCCAGGAA 7800
TAGGCCCTGG AATGGCCAAC CATAACCAGT TCCAGCAACC CCAAGGAGTT GGCTACCCAC 7860
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AGCAGCATAT GCTCCCAAAT CAGGCCCAGT CCCCACACCT ACAAGGCCAG CAGATCCCTA 8100
ATTCTCTCTC CAATCAAGTG CGCTCTCCCC AGCCTGTCCC TTCTCCACGG CCACAGTCCC 8160
AGCCCCCCCA CTCCAGTCCT TCCCCAAGGA TGCAGCCTCA GCCTTCTCCA CACCACGTTT 8220
CCCCACAGAC AAGTTCCCCA CATCCTGGAC TGGTAGCTGC CCAGGCCAAC CCCATGGAAC 8280
AAGGGCATTT TGCCAGCCCG GACCAGAATT CAATGCTTTC TCAGCTTGCT AGCAATCCAG 8340
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ACTTGAATTC AAACCTCTCA CAGAGTACAC TAGACATACA CTAGAGACAC CTTGTATTTT 8460
GGGAGCAAAA AAATTATTTT CTCTTAACAA GACTTTTTGT ACTGAAAACA ATTTTTTTGA 8520
ATCTTTCGTA GCCTAAAAGA CAATTTTCCT TGGAACACAT AAGAACTGTG CAGTAGCCGT 8580
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AATAGGTTTT ATTATTTTTT TTAAATTAAT GAACATATGT AATATTAATG AACATATGTA 8820
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TTATGGAAGA GTTAAAACAT TTCTAAACCA GAGGACAAAA GGGGTTAATG TTACTTTGAA 8940

ATTACATTCT ATATATATAT AAATATATAT AAATATATAT TAAAATACCA GTTTTTTTTC 9000
TCTGGGTGCA AAGATGTTCA TTCTTTTAAA AAATGTTTAA AAAAAA 9046

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7326 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGGCCGAGA ACTTGCTGGA CGGACCGCCC AACCCCAAAC GAGCCAAACT CAGCTCGCCC 60
GGCTTCTCCG CGAATGACAA CACAGATTTT GGATCATTGT TTGACTTGGA AAATGACCTT 120
CCTGATGAGC TGATCCCCAA TGGAGAATTA AGCCTTTTAA ACAGTGGGAA CCTTGTTCCA 180
GATGCTGCGT CCAAACATAA ACAACTGTCA GAGCTTCTTA GAGGAGGCAG CGGCTCTAGC 240
ATCAACCCAG GGATAGGCAA TGTGAGTGCC AGCAGCCCTG TGCAACAGGG CCTTGGTGGC 300
CAGGCTCAGG GGCAGCCGAA CAGTACAAAC ATGGCCAGCT TAGGTGCCAT GGGCAAGAGC 360
CCTCTGAACC AAGGAGACTC ATCAACACCC AACCTGCCCA AACAGGCAGC CAGCACCTCT 420
GGGCCCACTC CCCCTGCCTC CCAAGCACTG AATCCACAAG CACAAAAGCA AGTAGGGCTG 480
GTGACCAGTA GTCCTGCCAC ATCACAGACT GGACCTGGGA TCTGCATGAA TGCTAACTTC 540
AACCAGACCC ACCCAGGCCT TCTCAATAGT AACTCTGGCC ATAGCTTAAT GAATCAGGCT 600
CAACAAGGGC AAGCTCAAGT CATGAATGGA TCTCTTGGGG CTGCTGGAAG AGGAAGGGGA 660
GCTGGAATGC CCTACCCTGC TCCAGCCATG CAGGGGGCCA CAAGCAGTGT GCTGGCGGAG 720
ACCTTGACAC AGGTTTCCCC ACAAATGGCT GGCCATGCTG GACTAAATAC AGCACAGGCA 780
GGAGGCATGA CCAAGATGGG AATGACTGGT ACCACAAGTC CATTTGGACA ACCCTTTAGT 840
CAAACTGGAG GGCAGCAGAT GGGAGCCACT GGAGTGAACC CCCAGTTAGC CAGCAAACAG 900
AGCATGGTCA ATAGTTTACC TGCTTTTCCT ACAGATATCA AGAATACTTC AGTCACCACT 960
GTGCCAAATA TGTCCCAGTT GCAAACATCA GTGGGAATTG TACCCACACA AGCAATTGCA 1020
ACAGGCCCCA CAGCAGACCC TGAAAAACGC AAACTGATAC AGCAGCAGCT GGTTCTACTG 1080
CTTCATGCCC ACAAATGTCA GAGACGAGAG CAAGCAAATG GAGAGGTTCG NGCCTGTTCT 1140
CTCCCACACT GTCGAACCAT GAAAAACGTT TTGAATCACA TGACACATTG TCAGGCTCCC 1200
AAAGCCTGCC AAGTTGCCCA TTGTGCATCT TCACGACAAA TCATCTCTCA TTGGAAGAAC 1260
TGCACACGAC ATGACTGTCC TGTTTGCCTC CCTTTGAAAA ATGCCAGTGA CAAGCGAAAC 1320
CAACAAACCA TCCTGGGATC TCCAGCTAGT GGAATTCAAA ACACAATTGG TTCTGTTGGT 1380
GCAGGGCAAC AGAATGCCAC TTCCTTAAGT AACCCAAATC CCATAGACCC CAGTTCCATG 1440
CAGCGGGCCT ATGCTGCTCT AGGACTCCCC TACATGAACC AGCCTCAGAC GCAGCTGCAG 1500
CCTCAGGTTC CTGGCCAGCA ACCAGCACAG CCTCCAGCCC ACCAGCAGAT GAGGACTCTC 1560
AATGCCCTAG GAAACAACCC CATGAGTGTC CCAGCAGGAG GAATAACAAC AGATCAACAG 1620
CCACCAAACT TGATTTCAGA ATCAGCTCTT CCAACTTCCT TGGGGGCTAC CAATCCACTG 1680
ATGAATGATG GTTCAAACTC TGGTAACATT GGAAGCCTCA GCACGATACC TACAGCAGCG 1740
CCTCCTTCCA GCACTGGTGT TCGAAAAGGC TGGCATGAAC ATGTGACTCA GGACCTACGG 1800
AGTCATCTAG TCCATAAACT CGTTCAAGCC ATCTTCCCAA CTCCAGACCC TGCAGCTCTG 1860
AAAGATCGCC GCATGGAGAA CCTGGTTGCC TATGCTAAGA AAGTGGAGGG AGACATGTAT 1920
GAGTCTGCTA ATAGCAGGGA TGAATACTAT CATTTATTAG CAGAGAAAAT CTATAAAATA 1980
CAAAAAGAAC TAGAAGAAAA GCGGAGGACA CGTTTACATA AGCAAGGCAT CCTGGGTAAC 2040
CAGCCAGCTT TACCAGCTTC TGGGGCTCAG CCCCCTGTGA TTCCACCAGC CCAGTCTGTA 2100
AGACCTCCAA ATGGGCCCCT GCCTTTGCCA GTGAATCGCA TGCAGGTTTC TCAAGGGATG 2160
AATTCATTTA ACCCAATGTC CCTGGGAAAC GTCCAGTTGC CACAGGCACC CATGGGACCT 2220
CGTGCAGCCT CCCCTATGAA CCACTCTGTG CAGATGAACA GCATGGCCTC AGTTCCGGGT 2280
ATGGCCATTT CTCCTTCACG GATGCCTCAG CCTCCAAATA TGATGGGCAC TCATGCCAAC 2340
AACATTATGG CCCAGGCACC TACTCAGAAC CAGTTTCTGC CACAGAACCA GTTTCCATCA 2400
TCCAGTGGGG CAATGAGTGT GAACAGTGTG GGCATGGGGC AACCAGCAGC CCAGGCAGGT 2460
GTTTCACAGG GTCAGGAACC TGGAGCTGCT CTCCCTAACC CTCTGAACAT GCTGGCACCC 2520
CAGGCCAGCC AGCTGCCTTG CCCACCAGTG ACACAGTCAC CATTGCACCC GACTCCACCT 2580
CCTGCTTCCA CAGCTGCTGG CATGCCCTCT CTCCAACATC CAACGGCACC AGGAATGACC 2640
CCTCCTCAGC CAGCAGCTCC CACTCAGCCA TCTACTCCTG TGTCATCTGG GCAGACTCCT 2700
ACCCCAACTC CTGGCTCAGT GCCCAGCGCT GCCCAAACAC AGAGTACCCC TACAGTCCAG 2760
GCAGCAGCAC AGGCTCAGGT GACTCCACAG CCTCAGACCC CAGTGCAGCC ACCATCTGTG 2820
GCTACTCCTC AGTCATCACA GCAGCAACCA ACGCCTGTGC ATACTCAGCC ACCTGGCACA 2880
CCGCTTTCTC AGGCAGCAGC CAGCATTGAT AATAGAGTCC CTACTCCCTC CACTGTGACC 2940

AGTGCTGAAA CCAGTTCCCA GCAGCCAGGA CCCGATGTGC CCATGCTGGA AATGAAGACA 3000
GAGGTGCAGA CAGATGATGC TGAGCCTGAA CCTACTGAAT CCAAGGGGGA ACCTCGGTCT 3060
GAGATGATGG AAGAGGATTT ACAAGGTTCT TCCCAAGTAA AAGAAGAGAC AGATACGACA 3120
GAGCAGAAGT CAGAGCCAAT GGAAGTAGAA GAAAAGAAAC CTGAAGTAAA AGTGGAAGCT 3180
AAAGAGGAAG AAGAGAACAG TTCGAACGAC ACAGCCTCAC AATCAACATC TCCTTCCCAG 3240
CCACGCAAAA AAATCTTTAA ACCCGAGGAG CTACGCCAGG CACTTATGCC AACTCTAGAA 3300
GCACTCTATC GACAGGACCC AGAGTCTTTG CCTTTTCGTC AGCCTGTAGA TCCTCAGCTC 3360
CTAGGAATCC CAGATTATTT TGATATAGTG AAGAATCCTA TGGACCTTTC TACCATCAAA 3420
CGAAAGCTGG ACACAGGGCA ATATCAAGAA CCCTGGCAGT ATGTGGATGA TGTCAGGCTT 3480
ATGTTCAACA ATGCGTGGCT ATATAATCGT AAAACGTCCC GTGTATATAA ATTTTGCAGT 3540
AAACTTGCAG AGGTCTTTGA ACAAGAAATT GACCCTGTCA TGCAGTCTCT TGGATATTGC 3600
TGTGGACGAA AGTATGAGTT CTCCCCACAG ACTTTGTGCT GTTACGGAAA GCAGCTGTGT 3660
ACAATTCCTC GTGATGCAGC CTACTACAGC TATCAGAATA GGTATCATTT CTGTGGGAAG 3720
TGTTTCACAG AGATCCAGGG CGAGAATGTG ACCCTGGGTG ACGACCCTTC CCAACCTCAG 3780
ACGACAATTT CCAAGGATCA ATTTGAAAAG AAGAAAAATG ATACCTTAGA TCCTGAACCT 3840
TTTGTTGACT GCAAAGAGTG TGGCCGGAAG ATGCATCAGA TTTGTGTTCT ACACTATGAC 3900
ATCATTTGGC CTTCAGGTTT TGTGTGTGAC AACTGTTTGA AGAAAACTGG CAGACCTCGG 3960
AAAGAAAACA AATTCAGTGC TAAGAGGCTG CAGACCACAC GATTGGGAAA CCACTTAGAA 4020
GACAGAGTGA ATAAGTTTTT GCGGCGCCAG AATCACCCTG AAGCTGGGGA GGTTTTTGTC 4080
AGAGTGGTGG CCAGCTCAGA CAAGACTGTG GAGGTCAAGC CGGGAATGAA GTCAAGGTTT 4140
GTGGATTCTG GAGAGATGTC GGAATCTTTC CCATATCGTA CCAAAGCACT CTTTGCTTTT 4200
GAGGAGATCG ATGGAGTCGA TGTGTGCTTT TTTGGGATGC ATGTGCAAGA TACGGCTCTG 4260
ATTGCCCCCC ACCAAATACA AGGCTGTGTA TACATATCTT ATCTGGACAG TATTCATTTC 4320
TTCCGGCCCC GCTGCCTCCG GACAGCTGTT TACCATGAGA TCCTCATCGG ATATCTCGAG 4380
TATGTGAAGA AATTGGTGTA TGTGACAGCA CATATTTGGG CCTGTCCCCC AAGTGAAGGA 4440
GATGACTATA TCTTTCATTG CCACCCCCCT GACCAGAAAA TCCCCAAACC AAAACGACTA 4500
CAGGAGTGGT ACAAGAAGAT GCTGGACAAG GCGTTTGCAG AGAGGATCAT TAACGACTAT 4560
AAGGACATCT TCAAACAAGC GAACGAAGAC AGGCTCACGA GTGCCAAGGA GTTGCCCTAT 4620
TTTGAAGGAG ATTTCTGGCC TAATGTGTTG GAAGAAAGCA TTAAGGAACT AGAACAAGAA 4680
GAAGAAGAAA GGAAAAAAGA AGAGAGTACT GCAGCGAGTG AGACTCCTGA GGGCAGTCAG 4740
GGTGACAGCA AAAATGCGAA GAAAAAGAAC AACAAGAAGA CCAACAAAAA CAAAAGCAGC 4800
ATTAGCCGCG CCAACAAGAA GAAGCCCAGC ATGCCCAATG TTTCCAACGA CCTGTCGCAG 4860
AAGCTGTATG CCACCATGGA GAAGCACAAG GAGGTATTCT TTGTGATTCA TCTGCATGCT 4920
GGGCCTGTTA TCAGCACTCA GCCCCCCATC GTGGACCCTG ATCCTCTGCT TAGCTGTGAC 4980
CTCATGGATG GGCGAGATGC CTTCCTCACC CTGGCCAGAG ACAAGCACTG GGAATTCTCT 5040
TCCTTACGCC GCTCCAAATG GTCCACTCTG TGCATGCTGG TGGAGCTGCA CACACAGGGC 5100
CAGGACCGCT TTGTTTATAC CTGCAATGAG TGCAAACACC ATGTGGAAAC ACGCTGGCAC 5160
TGCACTGTGT GTGAGGACTA TGACCTTTGT ATCAATTGCT ACAACACAAA GAGCCACACC 5220
CATAAGATGG TGAAGTGGGG GCTAGGCCTA GATGATGAGG GCAGCAGTCA GGGTGAGCCA 5280
CAGTCCAAGA GCCCCCAGGA ATCCCGGCGT CTCAGCATCC AGCGCTGCAT CCAGTCCCTG 5340
GTGCATGCCT GCCAGTGTCG CAATGCCAAC TGCTCACTGC CGTCTTGCCA GAAGATGAAG 5400
CGAGTCGTGC AGCACACCAA GGGCTGCAAG CGCAAGACTA ATGGAGGATG CCCAGTGTGC 5460
AAGCAGCTCA TTGCTCTTTG CTGCTACCAC GCCAAACACT GCCAAGAAAA TAAATGCCCT 5520
GTGCCCTTCT GCCTCAACAT CAAACATAAC GTCCGCCAGC AGCAGATCCA GCACTGCCTG 5580
CAGCAGGCTC AGCTCATGCG CCGGCGAATG GCAACCATGA ACACCCGCAA TGTGCCTCAG 5640
CAGAGTTTGC CTTCTCCTAC CTCAGCACCA CCCGGGACTC CTACACAGCA GCCCAGCACA 5700
CCCCAAACAC CACAGCCCCC AGCCCAGCCT CAGCCTTCAC CTGTTAACAT GTCACCAGCA 5760
GGCTTCCCTA ATGTAGCCCG GACTCAGCCC CCAACAATAG TGTCTGCTGG GAAGCCTACC 5820
AACCAGGTGC CAGCTCCCCC ACCCCCTGCC CAGCCCCCAC CTGCAGCAGT AGAAGCAGCC 5880
CGGCAAATTG AACGTGAGGC CCAGCAGCAG CAGCACCTAT ACCGAGCAAA CATCAACAAT 5940
GGCATGCCCC CAGGACGTGA CGGTATGGGG ACCCCAGGAA GCCAAATGAC TCCTGTGGGC 6000
CTGAATGTGC CCCGTCCCAA CCAAGTCAGT GGGCCTGTCA TGTCTAGTAT GCCACCTGGG 6060
CAGTGGCAGC AGGCACCCAT CCCTCAGCAG CAGCCGATGC CAGGCATGCC CAGGCCTGTA 6120
ATGTCCATGC AGGCCCAGGC AGCAGTGGCT GGGCCACGGA TGCCCAATGT GCAGCCAAAC 6180
AGGAGCATCT CGCCAAGTGC CCTGCAAGAC CTGCTACGGA CCCTAAAGTC ACCCAGCTCT 6240
CCTCAGCAGC AGCAGCAGGT GCTGAACATC CTTAAATCAA ACCCACAGCT AATGGCAGCT 6300
TTCATCAAAC AGCGCACAGC CAAGTATGTG GCCAATCAGC CTGGCATGCA GCCCCAGCCC 6360
GGACTTCAAT CCCAGCCTGG TATGCAGCCC CAGCCTGGCA TGCACCAGCA GCCTAGTTTG 6420
CAAAACCTGA ACGCAATGCA AGCTGGTGTG CCACGGCCTG GTGTGCCTCC ACCACAACCA 6480
GCAATGGGAG GCCTGAATCC CCAGGGACAA GCTCTGAACA TCATGAACCC AGGACACAAC 6540
CCCAACATGA CAAACATGAA TCCACAGTAC CGAGAAATGG TGAGGAGACA GCTGCTACAG 6600
CACCAGCAGC AGCAGCAGCA ACAGCAGCAG CAGCAGCAGC AACAACAAAA TAGTGCCAGC 6660
TTGGCCGGGG GCATGGCGGG ACACAGCCAG TTCCAGCAGC CACAAGGACC TGGAGGTTAT 6720

GCCCCAGCCA TGCAGCAGCA ACGCATGCAA CAGCACCTCC CCATCCAGGG CAGCTCCATG 6780
GGCCAGATGG CTGCTCCAAT GGGACAACTT GGCCAGATGG GGCAGCCTGG GCTAGGGGCA 6840
GACAGCACCC CTAATATCCA GCAGGCCCTG CAGCAACGGA TTCTGCAGCA GCAGCAGATG 6900
AAGCAACAAA TTGGGTCACC AGGCCAGCCG AACCCCATGA GCCCCCAGCA GCACATGCTC 6960
TCAGGACAGC CACAGGCCTC ACATCTCCCT GGCCAGCAGA TCGCCACATC CCTTAGTAAC 7020
CAGGTGCGAT CTCCAGCCCC TGTGCAGTCT CCACGGCCCC AATCCCAACC TCCACATTCC 7080
AGCCCGTCAC CACGGATACA ACCCCAGCCT TCACCACACC ATGTTTCACC CCAGACTGGA 7140
ACCCCTCACC CTGGACTCGC AGTCACCATG GCCAGCTCCA TGGATCAGGG ACACCTGGGG 7200
AACCCTGAAC AGAGTGCAAT GCTCCCCCAG CTGAATACCC CCAACAGGAG CGCACTGTCC 7260
AGTGAACTGT CCCTGGTTGG TGATACCACG GGAGACACAC TAGAAAAGTT TGTGGAGGGT 7320
TTGTAG 7326

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2499 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCACTTGTCA ATTAATCCAG CTTCCTTAAT TTTACTGAAG AAGAATTTCT CCAGGATATT 60
GGCACATTTG TAGTATTCAC TCTCAGGGGC GTTGTACTCT TTGCAATTGG TAAAGACTCG 120
CTGTAAGTCT GCCATGAATA ATTTCTTAGA CACGTAGTAC CTATTCTTGA GGCGTTCACT 180
CATGGTTTTG AGATCCATGG GGGACCTTAT AACTTCATAA TATCCTGGAG CTTCTGTTCT 240
CTTCACAGGT TCCATGAAGG GCCAAGCGCT TTGATGGCTC TTCACCTGCT GGAGGATGCT 300
CTTGAGCGTG CTGTAAAGCT GGTCAGGGTC TCTGGGCTCT TTACTTTTCT CTTTTCCACT 360
CGGTTTCCAG CCTGTCTCTC TAATTCCAGG AATGCTTTCT ATAGGAATCT GTCGAACTCC 420
ATCTTTAAAA CATGAAAGTC CAGGGTAAAC TTTTCGAATT TGTGCCTGTT TTCTTTCAAT 480
CAGTTTTTTA ATTATCTCCT TCTGCTTTTT AATGATGACA GAAAATTCTG TGTACGGGAT 540
CCGTGGATTT AGCTCACATC CCATTAAAGT GGCTCCTTCA TAATCCTTGA TATAGCCAAC 600
ATATTTGGTT TTAGGTATTT TAATTTCTTT GGAGAAACCC TGTTTCTTAA AGTATCCAAT 660
TGCATATTCA TCTGCATATG TGAGGAAGTT CAGGATGTCA TGCTTTATGT GATATTCTTT 720
CAAATGATTC ATCAGGTGTG TTCCATAGCC CTTGACTTGC TCATTTGAGG TTACAGCACA 780
GAAGACAATC TCTGTGAATC CTTGAGATGG GAACATACGG AAACAGATAC CACCAATAAC 840
ACGGCCATCT TTAATTAAAG CAAGGGTTTT GTGTTTCGGG TCAAAGACGA GCCGTGTGAT 900
GTATTCTTTT GGCATTCGGG GCAGCTGGTG GGAGAAAACG TTCTGTAGGC CAACCAGCCA 960
CATCAGGATC TTCTTGTTTG GTTTCTGGTT GAGGGAATTG CCAACCACGT GAAATTCAAT 1020
TACACCCCTG CGCTCTTCCA ACCTTGCCGC CTCATCCCTG GCCGAGTGTG CTGACAGAAA 1080
ATTGGTCTCT GGTCCAAGCA TTGCTGCAGG GTCCGTGATG GTAGACATAA CCTCGTTGAT 1140
TAATTCCATC GGAATATCCC CCATAACTCG GGGTTTCTTG GCCTCCTCCA GAACATGAGA 1200
ATCAGTCATT TTCCTCTTTT CTCCTGGGTT TGCCTCAAGT CCAGAAGAGG CTTTGCAGGC 1260
AGGACTGCTG CTCCCTGCGT TTGGCTGCTC AAGGGAAGAT GAGGTTGAAT TGTATGAAAT 1320
TGTCCCAGCC ACAGGAGGTG GATTGATAAC TGTTTGGATG CCTAGCTGGC TGGTTCTGGA 1380
AGAGGCTGAG AGAAAATCCT GATCCCAGAT GGGAGAGTTT TGACTATATA CTTCTTCTTC 1440
TAGCATGGAC AGAAATTTTG GGAAATGAGT GAGGATTAGA GTTCGTTTTT CAAGAGGCAG 1500
TTTATCTTTT TCCTGTCTTG CTTGTTCCAG GAGTTGTCGC CTCATAACAG TGAAGACCGA 1560
GCGAAGCAAT GTTCTCCCAA ACACCTGTGT GGTTTCGTAC CGAGGTAGAC TGTCGCAGAA 1620
CTGTGGCACG TTGCAGTAAC ACAGCCACCT TGTGTAGTTC TCTTTGTATC CAGAAATATC 1680
ATCATTGGGA GATCGCAGTC TTCGTTGAGA TGGTGCCTCC AGATGCCAAT AGTTGATGCG 1740
GTTTAGGAAC ATTTTTGCCA ACTCAACTAT TGTTTGCCTT TCTTTTGCTG GCAGGTGACT 1800
AAATTTGTAC TGCACAAAGT TATTCACACC CTGTTCAATG CTAGGTTTTT CAAATGGGGG 1860
TTTCTTTTCC AAAGAGCCTT CAACCACAGG TTTTCCTCTT TGTAAAATAG ACTTTCTCAA 1920
GAGCTTAAAT AGATAGAAAT AAACTTGTTT GGTATCTGCA TCTTCTTCCT TGTGGACACA 1980
GGTAAAGAGA TATTCCACAT CCAATACTAT TCCCAGGAGT CTGTTCATTT CTTCCTCTGA 2040
CACATTCTCC AGGTGGGAAA CATGAGCAGC TAGGGCATGG CTACAACTCC GACAGGATTC 2100
TGTTAGACTG ACAATTATTT GCTGCAGGTC GGCTCTGGGG GGAGTGGGTG AGGGGTTAGG 2160
GTTTTTCCAG CCATTACATT TACAAGACTC CTCGGCCTTG CAGGCGGAGT ACACTCCGAG 2220
TTTCTCCAGT TTCTTGGCCC GCGGAGCGGA GCGTAGTTGC GCTTTCTTCA CGGCGATTCG 2280
GGCCGAGCCA CCGCCTCCCG GTCCTTCGGC CGTGCCCGCT GCAGCCACTG CCGTCGCCGG 2340
ACCGCAGGCG CCCGAGCCCC CGGCGGCAGC GGCGCAGGGG GAGCCCTGCG GGGGCGCGGG 2400

CGGAAGCGCC GCAGGCTGCG GGGGCAGCGC CCCGGGCCCG GCCCCTGCCC CGGCTCCTGC 2460
CCCGCAGCCG CCCGGCCCGG CCCCGCCAGC CTCGGACAT 2499

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2442 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCACTTGTCA ATCAACCCTG CTTCCTTAAT TTTACTGAAG AAGAACTTCT CCAGGATGCT 60
GGCGCATTTG TAGTACTCGC TCTCGGGAGG GTTGTACTCC TTGCAGTTGG TGAACACTCG 120
TTGCAAGTCC GCCATGAATA ACTTCTTAGA CACATAGTAC CTGTTCCTGA GGCGTTCACT 180
CATGGTTTTC AGATCCATGG GGAACCTTAT AACTTCATAA TATCCCGGAG CTTCTGTTCT 240
CTTCACTGGT TCCATGAAAG GCCAAGCATT TGGATGGTTC TTCACCTGCT GCAGGATGTT 300
CTTGAGGGTG CTGTAAACGT GCTCAGGGTC TTTGGGCTCT TTACTTTTCT CTTTTCCACT 360
TGGTTTCCAG CCTGTCTCTC TGATTCCAGG AATGCTTTCT ATAGGAATCT GCCGAACTCC 420
ATCTTTGAAA CACGAAAGTC CAGGGTAGAC TTTTCGAATC TGGGCTTGTT TTCTTTCTAT 480
CAGCTTTTTA ATGATCTCCT TCTGCTTTTT AATGATGACA GAGAACTCTG TGTATGGGAT 540
CTGAGGGTTC AGCTCACATC CCATCAAAGT GGCCCCTTCA TAATCCTTGA TGTAGCCAAC 600
ATATTTGGTT TTAGGTATTT TGATTTCTTT GGAGAAACCC TGCTTCTTGA AATAGCCGAT 660
GGCATACTCA TCTGCATATG TGAGGAAGTT GAGGATCTCG TGCTTTATGT GGTATTCTTT 720
GAGATGGTTC ATCAGGTGGG TTCCATAGCC CTTGACTTGT TCATTTGAGG TTACTGCACA 780
GAAAACAATC TCTGTGAATC CCTGGGATGG AAACATCCGG AAACAGATAC CACCAATGAC 840
ACGGCCATCT TTAATTAAAG CAAGGGTTTT GTGTTTCGGG TCAAAGACGA GCCGTGTGAT 900
GTACTCTTTG GGCATTCTGG GCAGCTGGTG GGAAAACACA TTCTGGAGGC CCACGAGCCA 960
CATCAGGATC TTCTTGTTTG GTTTCTGGTT CAGGGAGTTG CCCACCACGT GGAATTCAAT 1020
GACACCCCTG CGTTCTTCCA GCCGTGCCGC CTCATCTCTG GCCGAATGGG CTGACAGAAA 1080
ATTGGTCTCT GGTCCAAGCA TCCCTGCAGG GTCTGTGATG GTAGACATGA CCTCATTGAT 1140
CAATTCCACG GGAATATCCC CCATCACTCG AGATCTCTTG GCCTCCTCGG GAGCATGAGA 1200
GTTGTTCATT TTCCTCTTTT CTCCCGGGTT TGCTTCAAGC CCAGAAGAGC CTCTGCATCC 1260
AGGACTTGTT CTCCCTCCAT TGATCTGCTC ATGGGAAGTT GAATTTGAAC TGAACAATGC 1320
TGTCCCAGTA ACAGGAGGAC TGATTACTGT TTGGATTCCT AGCGGGCTGG TTCTGGAAGA 1380
GGCTGAGAGA AAATCCTGAT CCCAGATAGG AGAATTTTGA CTATACACTT CTTCTTCCAA 1440
CATGGACAGA AACTTTGGGA AATGTGTGAG GATAAGCGTG CGTTTCTCAA GAGGCAGTTT 1500
GTCTTTTTTC TGTCTGGCTT GTTCCAAGAG CTGTCGTCTC ATGATGGTGA AGACCGAGCG 1560
AAGCAATGTT CTCCCAAACA CCTTTGTGGT TTCGTACCGA GGTAAGCTGT CACAGAACTG 1620
CGGTACATTG CAGTAGCACA ACCACCTTGT GTAGTTTTCC TTGTATCCAG AGATGTCATC 1680
ATTGGGAGAC CGTAGTCTCC GCTGAGATGG AGCCTCCAGA TGCCAGTAGT TGATGCGGTT 1740
CAGAAACATC TTGGCCAGCT CGATCGTTGT CTGCCTCTCT TTCGATGGCA AGTGACTAAA 1800
CTTGTACTGC ACGAAGTTGT TCACACCCTG TTCAATACTG GGCTTCTCAA ATGGCGGCTT 1860
CTTCTCCAAG GAGCCTTCAA CCACAGGTTT TCCTCTTTGT AAAATTGACT TTCTCAAGAG 1920
CTTGAATAGG TAGAAGTACA CTTGTTTGGT ATCTGCATCT TCTTCTTTGT GGACGCAGGT 1980
GAAGAGGTAC TCCACATCCA ACACAATTCC CAGGAGTCTG TCCATCTCTT CCTCTGACAC 2040
ATTCTCCAAG TGAGAAACGT GAGCAGCAAG GGCATGGCTA CAGCTTCGAC AGGATTCTGT 2100
CAAACTGACA ATTATCTGCT GGAGGTCTCC TCTTGGTGGA GTAGGAGAGG GGTTAGGGTT 2160
CTTCCAGCCA TTGCATTTAC AGGACTCCTC TGCCTTGCAG GCGGAGTACA CGCCGAGTTT 2220
CTCCAGCTTC TTCGCCCGCG GAGCAGAGCG CAACTGCGCC TTCTTCACGG CGATCCGGGC 2280
CGAGCCGCCT CCTCCCGGTC CCTCGGCGGT GCCCGCCGCG GCCACCGGCG TCGCTGGCCC 2340
GCAGGAAGCA GAGCTCCCGG CAGCGGTGGC CAGGGTCCGG GGGGAACCGT GCGGGGGCGC 2400
GGGAGGCAGT GCTGGGGACC CGGCCCCGCC AGCCTCGGCC AT 2442

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCCGCCAGCC TCGGACATGC 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCCGCCAGCC TCGGCCATGC 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2442 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATGGCCGAGG CTGGCGGGGC CGGGTCCCCA GCACTGCCTC CCGCGCCCCC GCACGGTTCC 60
CCCCGGACCC TGGCCACCGC TGCCGGGAGC TCTGCTTCCT GCGGGCCAGC GACGCCGGTG 120
GCCGCGGCGG GCACCGCCGA GGGACCGGGA GGAGGCGGCT CGGCCCGGAT CGCCGTGAAG 180
AAGGCGCAGT TGCGCTCTGC TCCGCGGGCG AAGAAGCTGG AGAAACTCGG CGTGTACTCC 240
GCCTGCAAGG CAGAGGAGTC CTGTAAATGC AATGGCTGGA AGAACCCTAA CCCCTCTCCT 300
ACTCCACCAA GAGGAGACCT CCAGCAGATA ATTGTCAGTT TGACAGAATC CTGTCGAAGC 360
TGTAGCCATG CCCTTGCTGC TCACGTTTCT CACTTGGAGA ATGTGTCAGA GGAAGAGATG 420
GACAGACCCC TGGGAATTGT GTTGGATGTG GAGTACCTCT TCACCTGCGT CCACAAAGAA 480
GAAGATGCAG ATACCAAACA AGTGTACTTC TACCTATTCA AGCTCTTGAG AAAGTCAATT 540
TTACAAAGAG GAAAACCTGT GGTTGAAGGC TCCTTGGAGA AGAAGCCGCC ATTTGAGAAG 600
CCCAGTATTG AACAGGGTGT GAACAACTTC GTGCAGTACA AGTTTAGTCA CTTGCCATCG 660
AAAGAGAGGC AGACAACGAT CGAGCTGGCC AAGATGTTTC TGAACCGCAT CAACTACTGG 720
CATCTGGAGG CTCCATCTCA GCGGAGACTA CGGTCTCCCA ATGATGACAT CTCTGGATAC 780
AAGGAAAACT ACACAAGGTG GTTGTGCTAC TGCAATGTAC CGCAGTTCTG TGACAGCTTA 840
CCTCGGTACG AAACCACAAA GGTGTTTGGG AGAACATTGC TTCGCTCGGT CTTCACCATC 900
ATGAGACGAC AGCTCTTGGA ACAAGCCAGA CAGAAAAAAG ACAAACTGCC TCTTGAGAAA 960
CGCACGCTTA TCCTCACACA TTTCCCAAAG TTTCTGTCCA TGTTGGAAGA AGAAGTGTAT 1020
AGTCAAAATT CTCCTATCTG GGATCAGGAT TTTCTCTCAG CCTCTTCCAG AACCAGCCCG 1080
CTAGGAATCC AAACAGTAAT CAGTCCTCCT GTTACTGGGA CAGCATTGTT CAGTTCAAAT 1140
TCAACTTCCC ATGAGCAGAT CAATGGAGGG AGAACAAGTC CTGGATGCAG AGGCTCTTCT 1200
GGGCTTGAAG CAAACCCGGG AGAAAAGAGG AAAATGAACA ACTCTCATGC TCCCGAGGAG 1260
GCCAAGAGAT CTCGAGTGAT GGGGGATATT CCCGTGGAAT TGATCAATGA GGTCATGTCT 1320
ACCATCACAG ACCCTGCAGG GATGCTTGGA CCAGAGACCA ATTTTCTGTC AGCCCATTCG 1380
GCCAGAGATG AGGCGGCACG GCTGGAAGAA CGCAGGGGTG TCATTGAATT CCACGTGGTG 1440
GGCAACTCCC TGAACCAGAA ACCAAACAAG AAGATCCTGA TGTGGCTCGT GGGCCTCCAG 1500
AATGTGTTTT CCCACCAGCT GCCCAGAATG CCCAAAGAGT ACATCACACG GCTCGTCTTT 1560
GACCCGAAAC ACAAAACCCT TGCTTTAATT AAAGATGGCC GTGTCATTGG TGGTATCTGT 1620
TTCCGGATGT TTCCATCCCA GGGATTCACA GAGATTGTTT TCTGTGCAGT AACCTCAAAT 1680
GAACAAGTCA AGGGCTATGG AACCCACCTG ATGAACCATC TCAAAGAATA CCACATAAAG 1740
CACGAGATCC TCAACTTCCT CACATATGCA GATGAGTATG CCATCGGCTA TTTCAAGAAG 1800
CAGGGTTTCT CCAAAGAAAT CAAAATACCT AAAACCAAAT ATGTTGGCTA CATCAAGGAT 1860
TATGAAGGGG CCACTTTGAT GGGATGTGAG CTGAACCCTC AGATCCCATA CACAGAGTTC 1920
TCTGTCATCA TTAAAAAGCA GAAGGAGATC ATTAAAAAGC TGATAGAAAG AAAACAAGCC 1980
CAGATTCGAA AAGTCTACCC TGGACTTTCG TGTTTCAAAG ATGGAGTTCG GCAGATTCCT 2040

ATAGAAAGCA TTCCTGGAAT CAGAGAGACA GGCTGGAAAC CAAGTGGAAA AGAGAAAAGT 2100
AAAGAGCCCA AAGACCCTGA GCACGTTTAC AGCACCCTCA AGAACATCCT GCAGCAGGTG 2160
AAGAACCATC CAAATGCTTG GCCTTTCATG GAACCAGTGA AGAGAACAGA AGCTCCGGGA 2220
TATTATGAAG TTATAAGGTT CCCCATGGAT CTGAAAACCA TGAGTGAACG CCTCAGGAAC 2280
AGGTACTATG TGTCTAAGAA GTTATTCATG GCGGACTTGC AACGAGTGTT CACCAACTGC 2340
AAGGAGTACA ACCCTCCCGA GAGCGAGTAC TACAAATGCG CCAGCATCCT GGAGAAGTTC 2400
TTCTTCAGTA AAATTAAGGA AGCAGGGTTG ATTGACAAGT GA 2442

What is claimed is:

1. A purified protein designated P/CAF having a molecular weight of about 93,000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and which acetylates histones.

2. The protein of claim 1 consisting of the amino acid sequence of SEQ ID NO:1.

3. The protein of claim 1 comprising the amino acid sequence of SEQ ID NO:2.

4. The protein of claim 1, which also binds to the amino acid sequence of SEQ ID NO:3 on a p300 cellular protein and to amino acid residues 1805-1854 of a CBP cellular protein (SEQ ID NO:9).

5. A fragment of the protein of claim 1 having histone acetyltransferase activity.

6. A polypeptide consisting of the amino acid sequence of SEQ ID NO: 2.

7. A fragment of the protein of claim 1 which binds to the amino acid sequence of SEQ ID NO: 3 on the p300 cellular protein and the amino acid sequence of SEQ ID NO:9 on the CBP cellular protein.

8. A polypeptide consisting of the amino acid sequence of SEQ ID NO:4.

9. A nucleic acid consisting of the nucleotide sequence of SEQ ID NO:10.

10. A nucleic acid having a nucleotide sequence which encodes the protein of claim 1.

11. A nucleic acid having a nucleotide sequence which encodes the protein of claim 2.

12. A nucleic acid having a nucleotide sequence which encodes the protein of claim 3.

13. A nucleic acid consisting of the nucleotide sequence which encodes the protein of claim 4.

14. A nucleic acid complementary to and which selectively hybridizes with the nucleic acid of claim 11 under stringent hybridization conditions.

15. A fragment of the nucleic acid of claim 9, which encodes a polypeptide that acetylates histones.

16. A fragment of the nucleic acid of claim 9, which encodes a polypeptide which binds to the amino acid sequence of SEQ ID NO:3 on the p300 cellular protein and the amino acid sequence of SEQ ID NO:9 on the CBP cellular protein.

17. A purified antibody which specifically binds the protein of claim 1.

18. A purified antibody which specifically binds the protein of claim 2.

19. A purified antibody which specifically binds the protein of claim 3.

20. A purified antibody which specifically binds the protein of claim 4.

21. An assay for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of P/CAF comprising:

a) contacting the substance with a system in which histone acetylation by P/CAF can be determined;

b) determining the amount of histone acetylation by P/CAF in the presence of the substance; and

c) comparing the amount of histone acetylation by P/CAF in the presence of the substance with the amount of histone acetylation by P/CAF in the absence of the substance, a decreased or increased amount of histone acetylation by P/CAF in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of P/CAF.

22. An assay for screening substances for the ability to inhibit binding of P/CAF to p300/CBP comprising:

a) contacting the substance with a system in which the P/CAF binding of P300/CBP can be determined;

b) determining the amount of P/CAF binding of p300/CBP in the presence of the substance; and

c) comparing the amount of binding of P/CAF to p300/CBP in the presence of the substance with the amount of binding of P/CAF to p300/CBP in the absence of the substance, a decreased amount of binding of P/CAF to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of P/CAF to p300/CBP.

23. The method of claim 22, wherein the system consists of a cell free reaction mixture comprising a fragment of the p300 protein comprising amino acid residues 1767-1816 (SEQ ID NO:3) and the protein of claim 4.

24. The method of claim 22, wherein the system consists of a cell free reaction mixture comprising a fragment of the CBP protein comprising amino acid residues 1805-1854 (SEQ ID NO:9) and the protein of claim 4.

25. The method of claim 22, wherein the system consists of a cell extract produced from cells producing both p300 and P/CAF.

26. An assay for screening substances for the ability to inhibit or stimulate the histone acetyltransferase activity of p300/CBP comprising:

a) contacting the substance with a system in which histone acetylation by p300/CBP can be determined;

b) determining the amount of histone acetylation by p300/CBP in the presence of the substance; and

c) comparing the amount of histone acetylation by p300/CBP in the presence of the substance with the amount of histone acetylation by p300/CBP in the absence of the substance, a decreased or increased amount of histone acetylation by p300/CBP in the presence of the substance indicating a substance that can inhibit or stimulate, respectively, the histone acetyltransferase activity of p300/CBP.

27. An assay for screening substances for the ability to inhibit binding of a DNA-binding transcription factor to p300/CBP comprising:

a) contacting the substance with a system in which the DNA-binding transcription factor binding of P300/CBP can be determined;

b) determining the amount of DNA-binding transcription factor binding of p300/CBP in the presence of the substance; and

c) comparing the amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance with the amount of binding of DNA-binding transcription factor to p300/CBP in the absence of the substance, a decreased amount of binding of DNA-binding transcription factor to p300/CBP in the presence of the substance indicating a substance that can inhibit the ability to inhibit binding of DNA-binding transcription factor to p300/CBP.

28. The method of claim 27, wherein the system consists of a cell free reaction mixture comprising a DNA-binding transcription factor and p300/CBP.

29. The method of claim 27, wherein the system consists of a cell free reaction mixture comprising a fragment of the CBP protein comprising a DNA-binding transcription factor and p300/CBP.

30. The method of claim 27, wherein the system consists of a cell extract produced from cells producing both a DNA-binding transcription factor and p300/CBP.

31. The method of claim 27, wherein the DNA-binding transcription factor is selected from the group consisting of a nuclear hormone receptor, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YY1, Sap-1a, c-Fos, MyoD and SRC-1.

32. A method for inhibiting the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

33. The method of claim 32, wherein the substance can inhibit the transcription modulating activity of P/CAF by preventing the binding of P/CAF to p300/CBP.

34. A method for stimulating the transcription modulating activity of P/CAF in a subject, comprising administering to the subject a transcription modulating activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

35. The method of claim 34, wherein the substance can stimulate the transcription modulating activity of P/CAF by promoting the binding of P/CAF to p300/CBP.

36. The method of claim 34, wherein the substance can stimulate the transcription modulating activity of P/CAF by stimulating the histone acetlytransferase activity of P/CAF.

37. A method for inhibiting the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity inhibiting amount of a substance in a pharmaceutically acceptable carrier.

38. The method of claim 37, wherein the substance can inhibit the transcription modulating activity of p300/CBP by preventing the binding of a DNA-binding transcription factor to p300/CBP.

39. The method of claim 38, wherein the DNA-binding transcription factor is selected from the group consisting of a nuclear hormone receptor, CREB, c-Jun/v-Jun, c-Myb/v-Myb, YY1, Sap-1a, c-Fos, MyoD and SRC-1.

40. The method of claim 37, wherein the substance is an antibody which binds p300/CBP.

41. A method for stimulating the histone acetyltransferase activity of p300/CBP in a subject, comprising administering to the subject a histone acetyltransferase activity stimulating amount of a substance in a pharmaceutically acceptable carrier.

42. The method of claim 41, wherein the substance can stimulate the histone acetyltransferase activity of p300/CBP by promoting the binding of a DNA-binding transcription factor to p300/CBP.

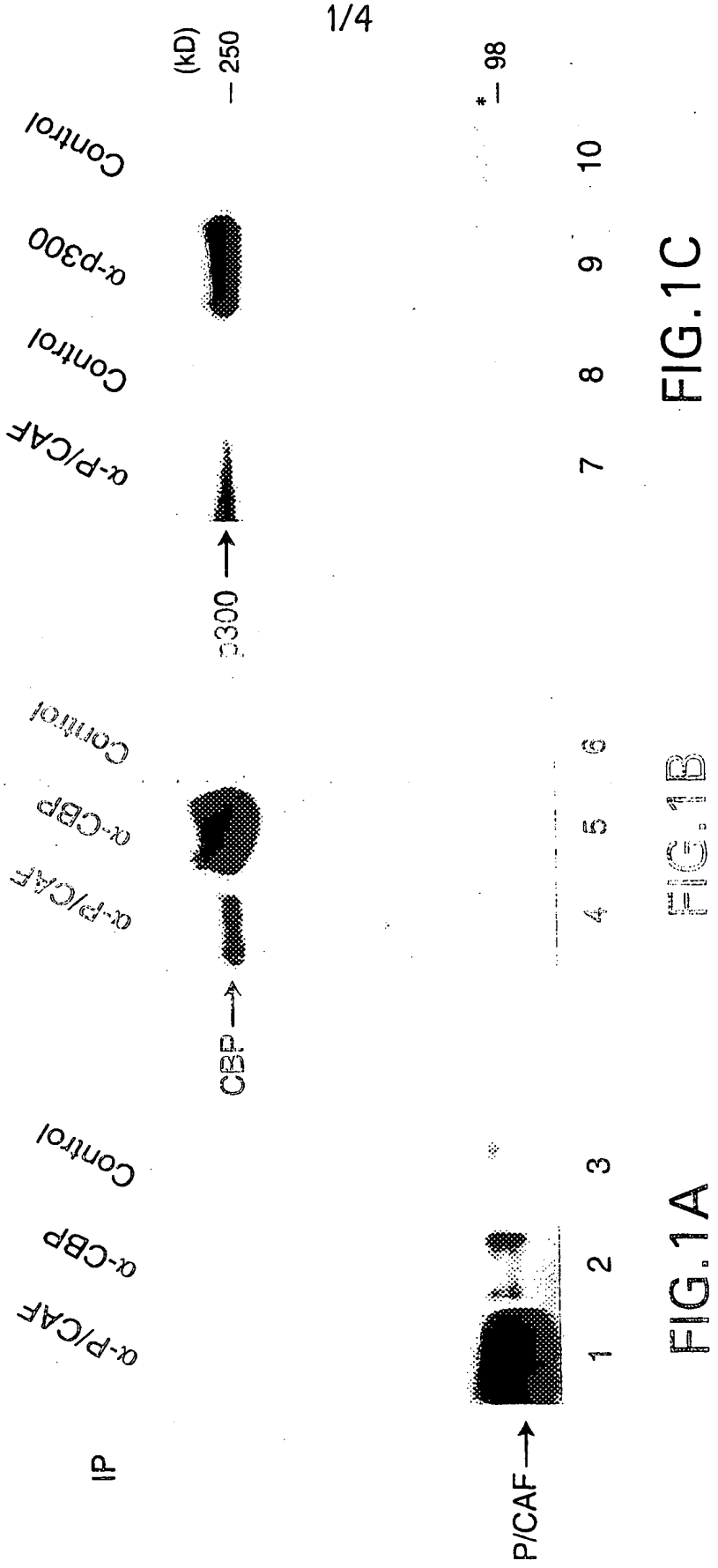

FIG. 1A

FIG. 1B

FIG. 1C

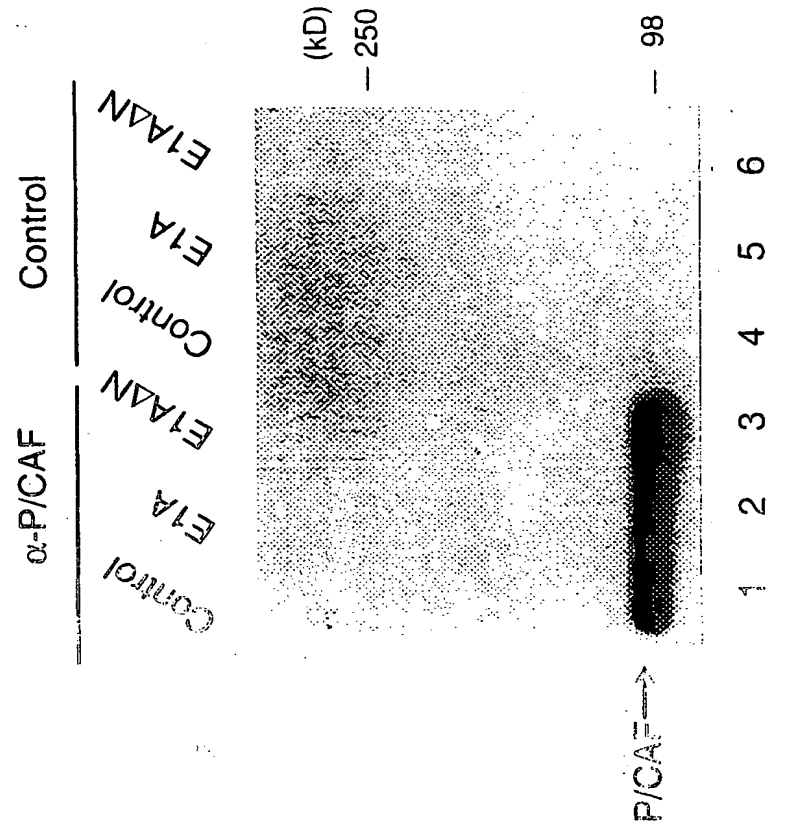
(kD)
250
98
Control
α-P/CAF
Control
E1A
E1ΔN
Control
E1A
E1ΔN
P/CAF
1 2 3 4 5 6

FIG.1E

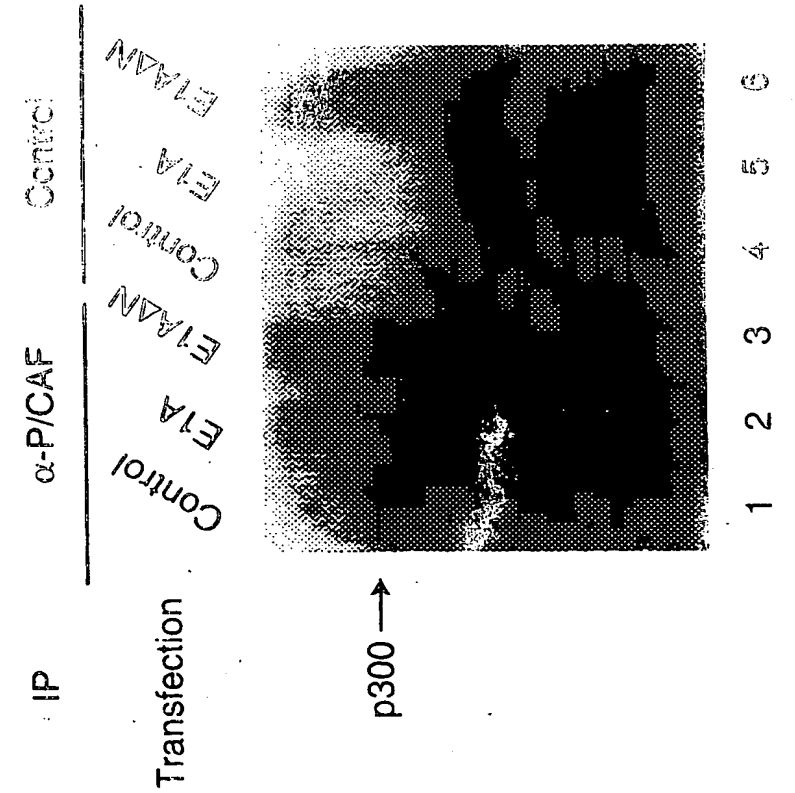
IP
Transfection
α-P/CAF
Control
E1A
E1ΔN
Control
E1A
E1ΔN
p300
1 2 3 4 5 6

FIG.1D

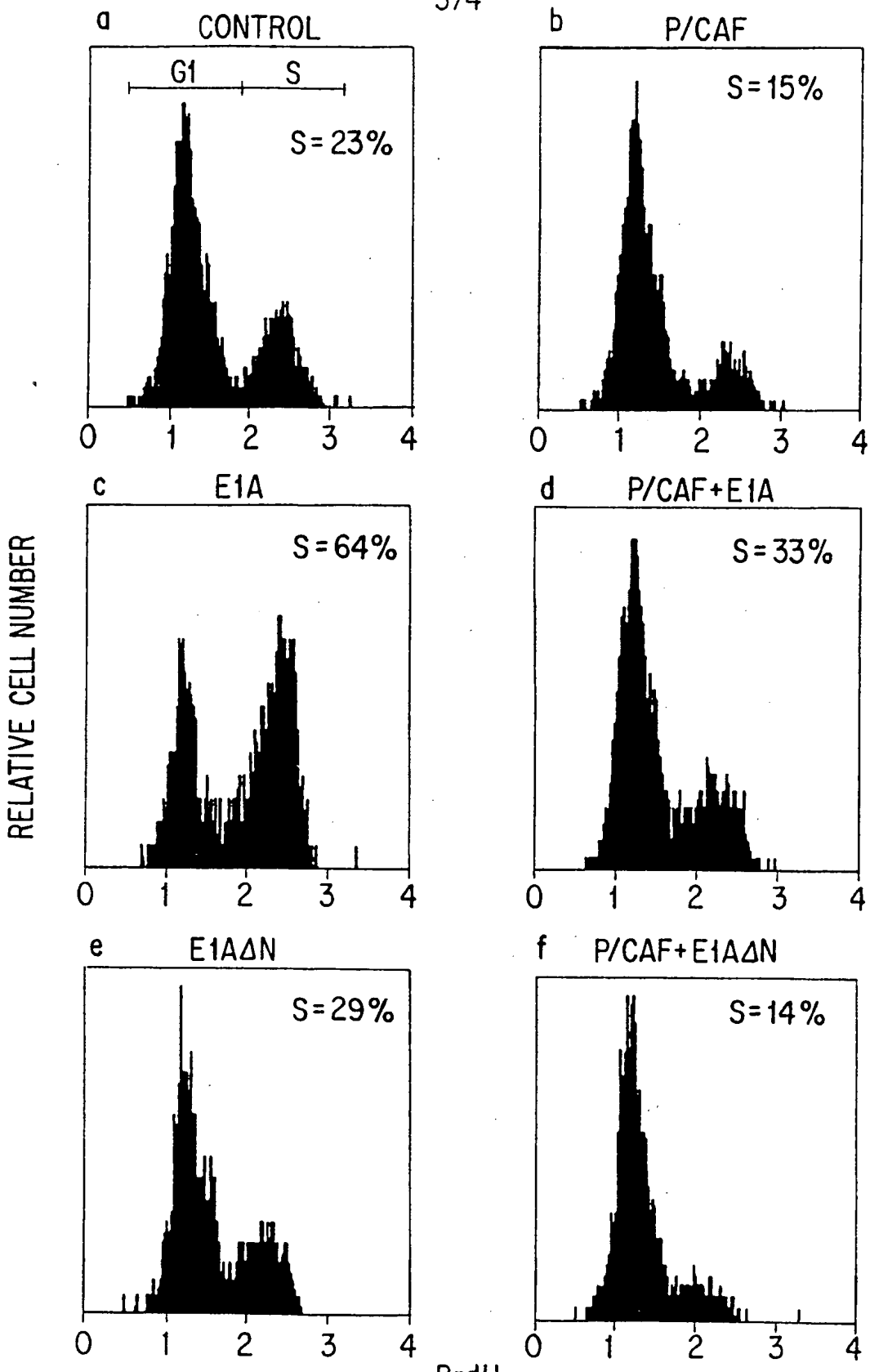
a
CONTROL
G1
S
S=23%
b
P/CAF
S=15%
c
E1A
S=64%
d
P/CAF+E1A
S=33%
e
E1AΔN
S=29%
f
P/CAF+E1AΔN
S=14%
RELATIVE CELL NUMBER
BrdU
0
1
2
3
4

FIG. 2

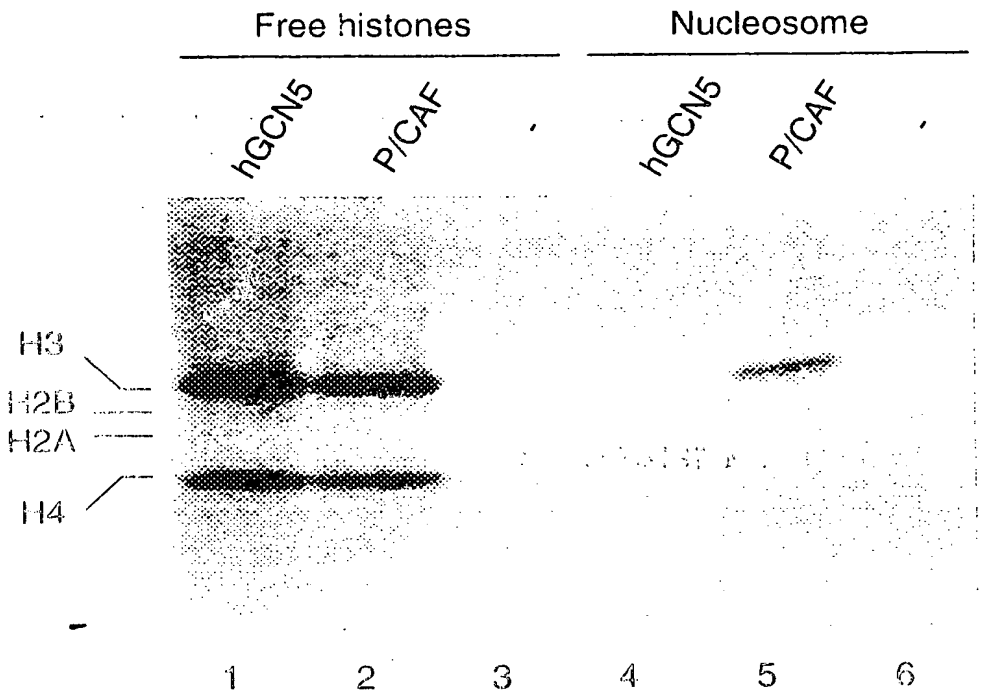
Free histones
Nucleosome
hGCN5
P/CAF
-
hGCN5
P/CAF
-
H3
H2B
H2A
H4
1
2
3
4
5
6

FIG.3

INTERNATIONAL SEARCH REPORT

Inter[redacted]nal Application No
PCT/US 97/12877

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 G01N33/50 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMBL EST, Accession numberN39522 Sequence no yv27b08.s1 Homo sapiens cDNA clone 243927 3' 25 January 1996 XP002050402 see the whole document ---	1
A	GEORGAKOPOULOS, T. & THIREOS, G.: EMBO JOURNAL., vol. 11, 1992, EYNSHAM, OXFORD GB, pages 4145-4152, XP002050399 see the whole document ---	1

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1997	14. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chambonnet, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/12877

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	YANG, X.Y. ET AL.: "A p300-CBP-associated factor that competes with the adenoviral oncoprotein E1A" NATURE., vol. 382, no. 8589, 25 July 1996, LONDON GB, pages 319-324, XP002050400 see the whole document	1
P,X	OGRYZKO, V.V. ET AL.: "The transcriptional coactivators p300 and CBP are histone acetyltransferases" CELL, vol. 87, no. 5, November 1996, NA US, pages 953-959, XP002050401 see the whole document	1
P,X	EMBL EST, Accession number U57316, Sequence reference human GCN5 (hGCN5) complete cds. 26 august 1996 XP002050403 see the whole document	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/12877

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 32 to 42 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the composition.